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STUDIES IN BACTERIOPHAGE*

I. THE BEHAVIOR OF THE BACTERIOPHAGE AND THE BACTERIA IN THE LESION AFTER THE TREATMENT OF ACUTE STAPHYLOCOCCUS SKIN INFECTIONS WITH BACTERIOPHAGE

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INTRODUCTION

IN THIS and in several subsequent papers we shall endeavor to report our laboratory observations in connection with the clinical use of bacteriophage. In reviewing the voluminous literature on the subject of bacteriolysis since the first reports by Twort[1] and d'Herelle[2] one finds that most of the articles fall into two groups: one confines itself to various aspects of the phenomenon in the test tube, while the other concerns itself purely with its clinical application. Only a few authors have reported experiences with the bacteriophage both in the clinic and laboratory and have attempted to set up controlled conditions for their observations. The reports of test tube phenomena have been contributed largely by investigators trying to work out some of the fundamental principles of the reaction, without attempting to apply them clinically. Their work has been of prime importance for our understanding of the nature of the phenomenon and for establishing a rationale for its use, but many of the phenomena which have been observed in vitro cannot be duplicated in vivo, and most of the reports of the clinical application of bacteriophage indicate that the results obtained have been poorly controlled by laboratory observations. The majority of clinical reports have been favorable.

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Failures have seldom been published. One does not persist in a method of treatment which does not have some degree of success. Therefore, only those who have had success have been able to collect a series of cases sufficiently large to be impressive, but it may be seriously questioned whether bacteriophage actually changed the course of some of the self-limited diseases which have been treated with it. The commercial exploitation of bacteriophage has resulted in its wide use by physicians who are not in a position to determine the potency of the products which they employ and who have made no attempt to set up control series sufficiently large to make it possible for them to properly evaluate their results.

We seriously question the wisdom of this uncontrolled use of bacteriophage at the present stage of our knowledge, because its successful use probably depends upon a combination of conditions intrinsic in the patient, in the bacterium concerned, and in the lytic principle available, as well as upon the manner in which these are brought together. Certain minimal essentials would seem to be required for success, a potent bacteriophage, a susceptible infecting organism, an adequate and intimate contact of bacteriophage with infecting organism, and an environment favorable to or at least not antagonistic to the interaction of these two. If the four conditions for success are absent or only partially fulfilled, bacteriolysis, in vivo, can hardly be expected to occur. It is not likely to occur with an impotent bacteriophage, with a resistant strain of bacteria, with poor contact of bacteriophage with organism or in an environment unfavorable or antagonistic to the interaction of these two. Whatever benefit may accrue from the injection or administration of the lytic products of bacteria as antigens has been given very little consideration. The whole procedure is still in the experimental stage and unless an effort is made to find out what the necessary conditions for success are and how to set them up, failure will be the rule and the whole subject will be brought generally into the disrepute in which it already finds itself, in the minds of some of the more conservative clinicians and bacteriologists. Extravagant claims for bacteriophage have been made largely by hyperenthusiasts or by those financially interested in its commercial success. This situation is to be deplored, for it seems fairly certain that in the hands of some critical investigators, favorable results have been obtained, results which at times have convinced both the physician and the patient that bacteriophage has some virtue as a method of therapy.

We have been impressed by incidents of this kind and have set out to clarify, if possible, certain obscurities with regard to the behavior of bacteriophage after its injection into the body and the behavior of the bacteria in the lesion after subjecting them to contact with the bacteriophage. In this paper we shall consider the following questions:

1. Does the hemolytic property of certain strains of *Staphylococcus aureus* bear any relationship to their resistance or to susceptibility to bacteriophage?

2. Do the bacteria in the lesion change their cultural characteristics after a single injection of bacteriophage?

3. Do the bacteria become more susceptible or more resistant to the action of bacteriophage?

4. Are the bacteria destroyed in the lesion by the action of bacteriophage?

5. Does the injected bacteriophage persist for any length of time in the lesion?

6. Does the injected bacteriophage increase in potency or amount in the lesion as d'Herelle has claimed[3]?

7. Does the bacteriophage injected at a site distant from the lesion reach the lesion through the circulation and multiply at the expense of the bacteria there?

8. Does the bacteriophage inhibiting substance (antiphage) found in the blood serum of some individuals inhibit the action of bacteriophage when injected into the lesion?

9. Can the apparently favorable effect of bacteriophage treatment in some cases be correlated with the absence of antiphage in the blood, the persistence of bacteriophage in the lesion or any other laboratory finding?

LITERATURE

The only reports bearing upon these questions which we can find in the recent literature are the following: In the treatment of a small group of twelve cases of urinary infections, Caldwell[4] found that after subcutaneous injection of bacteriophage it could be isolated from the urine. When bacteria disappeared from the urine following bacteriophage therapy the bacteriophage could no longer be recovered from the urine. She noted that the growth and pigment characteristics of the bacteria changed strikingly under bacteriophage action. In those cases in which the urine was not rendered bacteria-free following the use of bacteriophage the bacteria were modified and became resistant. The bacteriophage-resistant bacteria grew in smaller colonies than the original culture.

In 1926, Schumm and Cooke[5] looked for spontaneous bacteriophage in the washings of 40 cases of maxillary sinusitis and found it in four. In 11 of the negative cases, all of which were chronic, 1 c.c. of bacteriophage was instilled at weekly intervals, but no favorable results were obtained, although bacteriophage could subsequently be recovered from a majority of these cases and the organisms cultivated from the lesions remained susceptible to the bacteriophage in the test tube.

In 1926, Epstein and Fygin,[6] employing horse-blood agar, reported that 31 strains of hemolytic staphylococci were resistant to bacteriophage, while 91 nonhemolytic strains were susceptible. McKinley and Camara[7] (1930), working with a bacteriophage which was active against a strain of nonhemolytic staphylococcus, failed in their attempt to adapt it to 7 hemolytic strains. They believed that this observation tended to confirm the work of Epstein and Fygin, and suggested that this might explain the relative virulence of hemolytic organisms. Contrary results, however, were recently obtained by d'Herelle and Rakieten[8] who studied cultures from a hundred cases of staphylococcus infections, using human and rabbit blood agar. Seven strains of nonhemolytic staphylococci, isolated from chronic cases of furunculosis, sinusitis and osteomyelitis, were resistant to a number of polyvalent bacteriophages, while 71 strains of hemolytic staphylococci, with one exception, were found to be susceptible to the bacteriophage. The remaining 21 nonhemolytic strains were finally lysed, but in several instances only after repeated contact with the bacteriophage. These results were so at variance with the work of the preceding authors that d'Herelle and Rakieten repeated their experiments with horse-blood agar and found that the hemolytic strains were just as susceptible on that medium as they were on human and rabbit blood agar.

In 1931, Applebaum and McNeal[9] reported that purulent exudates and blood as well as serum, inhibited lysis sufficiently to explain the weakening of the action of bacteriophage and the persistence of living bacteria in purulent lesions treated with bacteriophage.

Colvin in 1932,[10] incidental to his observations on the inhibition of lysis by sterile pus, cerebrospinal fluid and serum, found that bacteriophage could be found in the lesion several days after its administration.

SOURCE OF MATERIAL AND METHOD OF STUDY

During the course of our study we have worked with four potent staphylococcus bacteriophages obtained from d'Herelle and Rakieten in New Haven;

from McNeal of the Post-Graduate Hospital, New York City; from Gratia in Brussels; and from Larkum of the Department of Public Health in Lansing, Michigan.

Our bacterial cultures were obtained from 110 patients with furuncles and carbuncles on their first visit to the Vanderbilt Clinic. If the lesion was draining, the exudate was collected on a swab, if not, a small amount of plain broth was injected into its center and reaspirated. This fluid was then cultivated for from three to five hours in plain broth. From the three- to five-hour growth in broth, four 5 c.c. tubes of plain broth were inoculated and to two of these tubes, 0.25 c.c. of bacteriophage was added. The other two tubes were kept as bacterial controls. One tube containing bacteriophage and one control were incubated at room temperature and one of each was incubated at body temperature for twenty-four hours. At the end of this time the tubes inoculated with bacteriophage were read for the degree of lysis and graded from 0 to 4+, the latter grade being assigned only to those tubes which were absolutely clear, comparison being made with the growth in the control tubes.

At the same time that the broth cultures were made, a drop from the control tube and a drop from the tube containing the culture and bacteriophage were smeared on the two halves of a sheep's blood agar plate. If the growth in the control tube at room temperature was less than the growth in the incubated control tube, it was filtered and the filtrate tested for the presence of spontaneous bacteriophage. Next day the plates were studied and the cultural characteristics of the colonies with and without bacteriophage were noted. If the colonies on the control half were rough or varied in size or shape or appeared "moth-eaten," spontaneous bacteriophage was suspected and searched for. When such cultures were found, transplants were made from these colonies into broth, grown at room temperature, filtered, and the filtrate was tested for lysis of the homologous and stock strains. In the more recent phases of our study, we have found spontaneous bacteriophage more often than we did at first because of greater care in the search.

At twenty-four, forty-eight, seventy-two, and ninety-six hours, after bacteriophage was given, cultures were again taken from the lesions and studied for the characteristics of the colonies and the susceptibility of the organisms to bacteriophage. Usually bacteriophage was given twenty-four hours after the first visit, the interval required to determine whether or not the bacteria were susceptible to bacteriophage.

RESULTS

I. *The Classification of* 110 *Strains of Staphylococcus Obtained from a Series of Furuncles and Carbuncles.*—In the whole series of 110 cultures, hemolytic *Staphylococcus aureus* occurred 81 times. In some of these cases the hemolysis was slight and could be seen only on removal of the colonies. Nonhemolytic *Staphylococcus aureus* was found in 18, and a mixture of these two in 8. Hemolytic *Staphylococcus albus* was found in one lesion and nonhemolytic *Staphylococcus albus* in one. Hemolytic *Staphylococcus aureus* and nonhemolytic *Staphylococcus albus* were combined in one. The results may be seen in Table I.

TABLE I

THE CLASSIFICATION OF 110 STRAINS OF STAPHYLOCOCCI ACCORDING TO THEIR HEMOLYTIC AND CHROMOGENIC PROPERTIES AND THEIR SUSCEPTIBILITY TO THE LYTIC ACTION OF BACTERIOPHAGE

	NO. OF CULT.	%	HEMOLYTIC STAPH. AUREUS	NONHEM. STAPH. AUREUS	BOTH HEMOLYTIC & NONHEM. STAPH. AUREUS	HEMOLYTIC STAPH. ALBUS	NONHEM. STAPH. ALBUS	HEMOLYTIC STAPH. AUREUS & NONHEM. S. ALBUS
Completely lysed	88	80	64	16	5	1	1	1
Partially lysed	19	17.3	15	1	3	–	–	–
Completely resistant	3	2.7	2	1	–	–	–	–
Totals	110	100	81	18	8	1	1	1

II. *The Susceptibility of the Hemolytic and Nonhemolytic Strains of Staphylococcus to Bacteriophage.*—Table I shows that only three of the 110 strains tested were found to be resistant to the action of all of the bacteriophages. Certain of the strains were resistant to one or two, but susceptible to at least one of the bacteriophages which we used. Two of the resistant strains were hemolytic *Staphylococcus aureus* and the other was a nonhemolytic *Staphylococcus aureus.* Of the 81 cultures of hemolytic *Staphylococcus aureus,* 64 were completely lysed; 15 underwent partial lysis; and 2 were resistant. Of the 18 strains of nonhemolytic *Staphylococcus aureus,* 16 underwent complete lysis; one partial lysis; and one was resistant. Of the 8 mixed cultures with both hemolytic and nonhemolytic *Staphylococcus aureus,* complete lysis took place in 5 and partial lysis in 3. With the 3 remaining albus cultures, either alone or with *Staphylococcus aureus,* complete lysis was obtained. In our series, therefore, no difference was found between the hemolytic and nonhemolytic strains in their susceptibility to bacteriophage.

III. *Persistence of Bacteriophage in the Lesion.*—The persistence of the bacteriophage in the lesion was studied in 32 patients, as the opportunity afforded upon their return to the clinic after treatment. In some the search was made after twenty-four hours and in others it was made later. In a few it was made daily, but in most of the cases we desired to institute daily treatment and did not withhold it to study the duration of the bacteriophage following a single injection. These results are shown in Table II. In 20, cultures were taken twenty-four hours after the first administration of potent bacteriophage. As a general rule, these cultures displayed considerable degeneration of the colonies on the blood agar plate. Often only remnants of colonies were left, or the intact colonies varied in size and shape. However, only rarely did we find no growth at all on the plate. On only two occasions, cultures of the lesion twenty-four hours after administration of bacteriophage yielded no growth on the plate or in broth. At the time of these observations, we assumed that bacteriophage was responsible, but its presence was not definitely proved. Broth tubes inoculated with cultures taken twenty-four hours after the administration of bacteriophage usually did not show growth when incubated at room temperature, but gave abundant growth

TABLE II

THE PERSISTENCE OF BACTERIOPHAGE 24, 48, 72, AND 96 HOURS AFTER INJECTION INTO THE LESION CORRELATED WITH THE CLINICAL EVIDENCE OF BENEFIT FOLLOWING THE TREATMENT

NUMBER	24 HR.	48 HR.	72 HR.	96 HR.	CLINICAL EFFECT
1		+			Slight benefit
2		+			Slight benefit
3	+	+			Slight benefit
4	+				Marked benefit
5	+	+			Marked benefit
6	+		0		Marked benefit
7	+			0	Marked benefit
8	0	0			No benefit
9		+		+	Marked benefit
10	+		+		Marked benefit
11				+	Marked benefit
12			+	0	Marked benefit
13		+			Slight benefit
14	0	0			Marked benefit
15	+	+	+		Marked benefit
16	+	+	+		Marked benefit
17	+	+		0	Marked benefit
18	+	+			Marked benefit
19		+			Marked benefit
20	+	+			Slight benefit
21	+				Marked benefit
22	+				Marked benefit
23			+		Marked benefit
24	+				Marked benefit
25	+				Marked benefit
26		0			No benefit
27	0	0	0		No benefit
28		0			Marked benefit
29		0			No benefit
30	0			0	No benefit
31			+		Marked benefit
32	+				Marked benefit
Totals	20	18	8	6	
Positive	16	12	6	2	
Negative	4	6	2	4	

in the incubator, although occasionally secondary lysis took place. Filtrates made of the 20 cultures left at room temperature produced complete lysis of the original homologous organisms obtained from the lesion in 16 instances, demonstrating the presence of potent bacteriophage. These bacteriophages often produced lysis of heterologous strains as well. They remained polyvalent and did not become specific for the homologous strain by passage. There is evidence, then, both from the appearance of the blood plates and from the test of the filtrates incubated at room temperature, that bacteriophage may be present in the lesion in about 4 cases out of 5 at least twenty-four hours after a single injection, and that under its influence there is a change in the growth characteristics and morphology of the organisms.

Cultures were taken from 18 cases forty-eight hours after the injection of bacteriophage. In most of these cases the culture was not taken after the first injection of bacteriophage, but later in the course of treatment. Therefore these and later determinations of bacteriophage in the lesion are not as significant as the tests made after twenty-four hours. The characteristics of the colonies on the blood plates made at this interval differed from those taken after twenty-four hours. Usually only a few colonies were af-

fected while the rest were normal. Large areas of destruction in the colonies were seldom observed. Degeneration of the organisms was not seen when these colonies were smeared and stained. Moreover, in the tubes incubated at room temperature some growth of organisms occurred in a high percentage of cases. When these tubes were filtered, however, the lytic principle was obtained from 12 of the 18, although the potency of the bacteriophage was definitely diminished. Those incubated at 37° C. usually showed abundant growth of bacteria.

It must be concluded, then, that bacteriophage persisted in the lesions for forty-eight hours in a smaller percentage of the cases and had less potency than at twenty-four hours.

Bacteriophage persisted in the lesion in 6 out of 8 cases tested after seventy-two hours, and in 2 out of 6 tested after ninety-six hours. The colonies on the blood plates showed no evidence of the destructive action, but the bacteriophages were recovered from the filtrates of cultures incubated at room temperature. These bacteriophages frequently gave only partial lysis, however.

IV. *The Relationship of the Persistence of Bacteriophage in the Lesion to the Presence of Antiphage in the Blood.*—In 22 cases we tried to correlate the persistence of bacteriophage in the lesion with the presence of an inhibiting substance in the blood serum. In testing for the presence of this substance we employed Rosenthal's technic.[11] In some instances the antiphage tests were done with the homologus strain of bacteria and in other cases with stock strains (Table III). Indirect antiphage (supposed to render the bacteria resistant to the lytic action of the bacteriophage) was only detected in 3 cases, while the direct antiphage (supposed to directly neutralize the action of the bacteriophage) was present in 16. Of 10 cases in which the test was made with the homologous strain, 7 showed a direct antiphage in the blood, but in 6 of these cases bacteriophage was found in the lesion from twenty-four to ninety-six hours after injection. The other 3 cases showed no direct antiphage in the blood, and bacteriophage was found in the lesion.

Of 11 cases in which the test was made for indirect antiphage using the homologous strain, two showed the presence of antiphage in blood, but the bacteriophage was later found in the lesion in both cases. In 9 cases no antiphage was found, but in 2 of these no bacteriophage could be found in the lesion.

Of 11 cases in which the test was made for direct antiphage, using a heterologous strain, 9 showed the presence of antiphage in the blood and yet in 7 of these bacteriophage was found in the lesion. In the 2 cases in which no antiphage was found in the blood, bacteriophage was present in the lesion. In the same 11 cases when the test was made for indirect antiphage using a heterologous strain, one showed the presence of antiphage in the blood, but bacteriophage was found in the lesion after twenty-four hours, while in 2 cases in which no antiphage was found in the blood, no bacteriophage could be found in the lesion after twenty-four hours. In 8 cases no antiphage was demonstrated in the blood and bacteriophage persisted in the lesion.

TABLE III

THE PRESENCE OF A BACTERIOPHAGE-INHIBITING SUBSTANCE, "ANTIPHAGE," IN THE BLOOD SERUM OF THE PATIENT CORRELATED WITH THE PERSISTENCE OF BACTERIOPHAGE IN THE WOUND FOLLOWING TREATMENT

NO.	ANTIPHAGE IN THE SERUM*			PERSISTENCE OF PHAGE IN THE LESION		
	STRAIN TESTED	DIRECT	INDIRECT	24 HOURS	48 HOURS	72 HOURS
1	Homol.	Strong	Weak	+	−	
2	Homol.	0	0	+		
3	Homol.	Weak	0	+		
4	Homol.	No test	0	0	0	
5	Homol.	Strong	0		+	
6	Homol.	Strong	0	+	+	+
7	Homol.	Strong	0	+	+	+
8	Homol.	0	0		+	
9	Homol.	Strong	0		0	
10	Homol.	0	0	+	+	
11	Homol.	Weak	Weak			+
12	Heterol.	Strong	0		+	
13	Heterol.	Strong	0	+		0
14	Heterol.	Weak	0	+	+	
15	Heterol.	Strong	0	+		
16	Heterol.	Weak	0			+
17	Heterol.	Strong	0		0	
18	Heterol.	Strong	0	0	0	0
19	Heterol.	0	0		+	
20	Heterol.	Strong	Strong	+		
21	Heterol.	0	0	+	+	
22	Heterol.	Weak	0	+		

*As determined by the Rosenthal technic.

V. *Transmission of Bacteriophage in the Patient from a Site Remote from the Lesion to the Lesion Itself.*—In 8 patients, satellite furuncles developed around the original lesion treated with bacteriophage. Attempts to isolate the bacteriophage from these new lesions failed. Again, 4 patients were given subcutaneous injections of large quantities of bacteriophage at a distance from the lesion and bacteriophage could only be isolated from one of the lesions twenty-four hours later. This bacteriophage was very weak, producing only 1+ lysis of the homologous organism, and it produced no lysis of two heterologous strains against which the injected bacteriophage had previously been effective. In this one case, no bacteriophage was evident in the lesion before the injection, so that we must presume that it had either been transmitted from the site of the injection or was spontaneously generated. With the exception of this one questionable instance, we have no evidence that bacteriophage can be transmitted within the body from a site distant from a skin lesion to the lesion itself.

VI. *Behavior of the Bacteria Which Survive in the Lesions Which Have Been Treated with Bacteriophage.*—Our experience has shown that the surviving progeny of a culture of hemolytic *Staphylococcus aureus* which has been lysed by bacteriophage but not entirely destroyed, produces nonhemolytic colonies when grown on blood agar plates. Usually the colonies at first become mixed, some being hemolytic and some nonhemolytic, but in a few instances in our series all of the colonies promptly became completely nonhemolytic. In vivo a similar change has occasionally been observed, but it required more prolonged contact of the bacteria with the bacteriophage. Of

28 cases studied, this transformation occurred in 7. In 5 of these, the hemolytic variety was replaced after one or more bacteriophage treatments by a mixture of the hemolytic and nonhemolytic strains. In another, the hemolytic strain was completely replaced by nonhemolytic organisms, while in the last case the culture yielded a mixture of hemolytic *Staphylococcus aureus* and nonhemolytic *Staphylococcus albus*. The possibility of contamination in this case is recognized.

We have never observed a transformation of the staphylococcus from nonhemolytic to hemolytic after lysis in the test tube by bacteriophage, but such a change was observed on three occasions in patients in the course of bacteriophage treatment. Two of these new strains became resistant to bacteriophage, whereas the original cultures were completely lysed before treatment began.

These cases also illustrate that bacteria may become resistant to the bacteriophage during treatment. Acquisition of resistance was observed in 6 out of 36 cases, and in 4 of these, cultures were taken as the lesions were healing. In 8 instances the bacteria became more susceptible to bacteriophage while in the remaining 22 cases no change in the susceptibility of the bacteria to the bacteriophage took place.

It was also observed, after bacteriophage had been injected into the lesion, that the colonies frequently changed from smooth to rough. Moreover, some of the colonies became pinpoint. No further changes than these could be detected in their characteristics as healing progressed in the presence of bacteriophage, except that some of the colonies from the larger carbuncles changed in color from yellow to white.

At no time could we surely demonstrate a complete destruction of bacteria in the lesion tested with repeated injections of bacteriophage. They persisted even after the slough and pus had disappeared. On several occasions, moreover, positive cultures were obtained after the removal of the terminal scab.

Bacteria also persisted in 7 lesions in which bacteriophage was generated spontaneously. These cultures showed considerable degeneration, however, and many of the colonies were roughened. All of the bacteriophages isolated from these lesions were polyvalent and possessed considerable potency.

From our results, therefore, we can only conclude that while the bacteriophage may change the characteristics of the bacteria in the lesion, it certainly does not completely destroy them.

VII. *The Correlation of Various Laboratory Findings With the Clinical Results.*—Untreated furuncles and carbuncles run a varied course under the variable conditions found in different individuals. Furuncles tend to be self-limited. There is a period of induration, a period of liquefaction and discharge, and a period of resolution. The same periods are more prolonged in carbuncles. The extent of the slough, the rapidity of liquefaction, and the size and number of the drainage openings all modify the period of spontaneous healing. With such variable controls it is very difficult to judge in any given cases whether or not any form of treatment hastens or delays the process of

healing. In order to furnish convincing evidence, we must await the acquisition of a large series of treated cases with an exactly parallel series of controls, unless we can apply a clear-cut laboratory criterion. This we are trying to establish. For the present we have tried to note from the appearance of the lesion after treatment, as well as from the subjective symptoms of the patient, whether or not the progress of the infection indicated a favorable or an indifferent effect from the bacteriophage treatment. (In no case have we had an unfavorable reaction.) We have tried to discover whether this could be correlated with (a) the presence of a bacteriophage-inhibiting substance, "antiphage," in the blood, or (b) the persistence of bacteriophage in the lesion, or (c) the maintenance of susceptibility to the bacteriophage on the part of the invading organism, or (d) changes in the hemolytic action of the cultures on blood agar.

(a) *Antiphage*: In 34 cases a study was made of the presence of antiphage in the blood with the Rosenthal technic,[11] and the tests were carried out with the homologous organisms (see Table IV). Eight of these cases gave evidence of the presence of both strong direct and strong indirect antiphage. After instituting bacteriophage treatment, our belief was that the bacteriophage had exerted a favorable influence in 5 of these and had had no influence on the infection in 3. Seven of the cases gave a strongly positive test for direct and a negative test for indirect antiphage. Of these, 5 gave a favorable response to bacteriophage, while 2 seemed to be slightly improved by it. Eight of the cases gave evidence of a weak direct antiphage and no sign of indirect antiphage in the blood; and of these, 5 gave a favorable response and 2 a questionable response, while one was not affected. Five cases gave no evidence of direct or indirect antiphage. Of these, 2 were favorably affected and 3 gave a questionable response. The other groups are too small to be significant.

TABLE IV

THE PRESENCE OF A BACTERIOPHAGE-INHIBITING SUBSTANCE IN THE BLOOD SERUM OF THE PATIENT CORRELATED WITH THE CLINICAL EFFECT OF PHAGE TREATMENT

NO. OF CASES	ANTIPHAGE IN SERUM*						CLINICAL EFFECT OF PHAGE TREATMENT		
	DIRECT			INDIRECT					
	STRONG	WEAK	NONE	STRONG	WEAK	NONE	BENEFIT	SLIGHT BENEFIT	NO BENEFIT
8	+			+			5	0	3
3	+				+		2	1	0
7	+					+	5	2	0
0		+		+			0	0	0
1		+			+		1	0	0
8		+				+	5	2	1
0			+	+			0	0	0
2			+		+		2	0	0
5			+			+	2	3	0

*As determined by Rosenthal's technic.

(b) *Persistence of Bacteriophage in the Lesion (see Table II)*: In the 32 cases in which the bacteriophage was sought for, twenty-four to ninety-six hours after its injection into the lesion, 16 cases out of 20 tested showed the bacteriophage in twenty-four hours. Of these, 14 gave a very favorable re-

sponse while 2 were slightly favorable. Of 4 cases which yielded no bacteriophage after twenty-four hours, one seemed to be favorably affected by the treatment while 3 were unaffected. The favorable case had already completely liquefied when treatment began. In forty-eight hours, 18 cases were tested for the persistence of bacteriophage. Twelve of these cases showed bacteriophage in the lesion at that time. Seven of them responded very favorably, while in 5 the effect was slightly favorable. Of 6 cases which showed no bacteriophage after forty-eight hours, 2 seemed to respond well after bacteriophage treatment, while 4 gave no evidence of any favorable response. One of the favorable cases had already liquefied. Out of 8 cases tested after seventy-two hours, bacteriophage was found in 6 and all seemed to be benefited by the bacteriophage treatment. In the 2 cases in which no bacteriophage was found after seventy-two hours, one seemed to be favorably affected by bacteriophage treatment while the other was not. In the favorable case, bacteriophage had been found in twenty-four hours. In 6 cases bacteriophage was sought for, ninety-six hours after its injection. It was found in 2 cases only. In 3 others it had been present at twenty-four, forty-eight, or seventy-two hours. In all of these cases, the result was favorable. In the other case in which it was not found, it had also been absent at forty-eight hours. In this case the effect of bacteriophage was negligible.

(c) *Maintenance of Susceptibility of the Bacteriophage on the Part of the Culture:* After treatment with bacteriophage, as we have stated above, the organism cultured from the lesion may show an increased or decreased susceptibility to the bacteriophage. This observation was correlated with the clinical response in twenty-eight cases (see Table V). In 25 of these cases the organism remained susceptible to the bacteriophage. Of these, 20 gave a favorable response while 5 were not benefited. In 2 cases the organism was only partially susceptible and these cases were not benefited. In 3 cases the organism became resistant to the bacteriophage and none of these was helped by the bacteriophage treatment.

TABLE V

THE MAINTENANCE OF SUSCEPTIBILITY TO PHAGE ON THE PART OF THE CULTURE CORRELATED WITH THE CLINICAL EFFECT OF BACTERIOPHAGE TREATMENT

	NO. OF CASES	CLINICAL EFFECT OF PHAGE TREATMENT	
		BENEFITED	NO BENEFIT
Remained susceptible	25	20	5
Became resistent	3	0	3

(d) *The Changes in the Hemolytic Action of the Culture on Blood Agar:* We have stated above that when hemolytic *Staphylococcus aureus* is lysed by bacteriophage in the test tube, if it is not entirely destroyed, the progeny are generally nonhemolytic. These bacteria are evidently resistant to the lytic effect of the bacteriophage. In treated cases these forms often appear, but not as regularly as in the test tube. The clinical result in these cases is not as favorable as in those in which the organism, which is recovered from

the lesion after treatment, is the same as it was before treatment whether it were hemolytic or nonhemolytic. In thirty-four cases in which this point was studied, 22 showed no change in the culture and the clinical results were favorable in 18 and indifferent in 4. In the other 12 cases there was some modification of the culture, consisting of a change either in the hemolytic or chromogenic function of the organism. In 4 of these the results were favor-

TABLE VI

CHANGES IN THE CULTURAL CHARACTERISTICS OF THE BACTERIAL STRAINS FOLLOWING BACTERIOPHAGE TREATMENT CORRELATED WITH THE CLINICAL RESULTS

	NO. OF CASES	CLINICAL EFFECT OF PHAGE TREATMENT		
		BENEFITED	SLIGHT BENEFIT	NO BENEFIT
No change in culture	22	18	0	4
Change in culture	12	4	2	6

able, in two there was partial success, either favorable at first and later indifferent, or indifferent at first and later favorable, while in 6 there was no apparent response to the treatment (see Table VI).

DISCUSSION

We recognize the difficulty of judging whether or not the administration of bacteriophage affects in any way the course of the disease. In order to be thoroughly convinced by clinical criteria alone, we should have two absolutely parallel series, one treated with bacteriophage and one untreated. We are at present attempting to assemble two such parallel series, taking into account the age of the lesion, its location, the presence and degree of necrosis, and the size of the lesion at the time of the beginning of treatment. If the results in these treated cases can be correlated with certain consistent laboratory findings, the latter may be depended upon as bases for prognosis.

It has been observed repeatedly that the most severe staphylococcus infections generally yield on culture a hemolytic strain of *Staphylococcus aureus*. Lesions somewhat less severe may yield a culture showing no hemolysis or a very slight degree of hemolysis beneath a few colonies on a blood plate. Still less severe lesions more often yield *Staphylococcus albus* rather than *S. aureus*. In our series the primary cultures yielded the usual proportion of such variable strains. The cultures which we called a mixture of hemolytic and nonhemolytic *Staphylococcus aureus* may have been pure cultures of the intermediate group, but usually those showing frankly yellow and frankly white colonies do represent a mixture of aureus and albus. It is rather surprising that in lesions of this kind mixtures are not more frequently found.

In our studies the hemolytic and nonhemolytic strains showed an equal susceptibility to the bacteriophages which we employed. Attention must be called to the fact, however, that our organisms were freshly isolated from acute stephylococcus infections and the same results might not have been obtained with staphylococci obtained from chronic infections or with cultures grown for some time in artificial media. Thus we did not confirm the observations of either d'Herelle and Rakieten or McKinley and Camara whose results

were opposed to one another. Differences in results could probably be explained by differences in the bacteriophages employed. This point does not seem to be important. There does seem to be some significance, however, in the fact that both in the test tube and in the patient the organisms undergo certain changes after contact with the bacteriophage. The hemolytic strains frequently become nonhemolytic, the smooth strains tend to become rough, and while they may become more resistant to the lytic action of the bacteriophage, both of these changes are in the direction of nonvirulence. It should be remembered that this occurred more consistently in the test tube than in the patient, but such changes occurring in the lesion might favor the prompt recovery from the infection by the action of the defensive forces of the body.

The persistence of the bacteriophage for twenty-four hours or more in the lesion after one injection is of considerable importance from a practical viewpoint. We do not feel that there is any evidence that it actually propagates itself there, and when injected elsewhere what may appear in the lesion gives no evidence of increasing either in quantity or in potency. In the lesion evidently something inhibits the multiplication of the bacteriophage, and yet it is not due to the presence of any inhibiting substance in the patient's serum, if Rosenthal's method can be relied upon, for the failure of the bacteriophage to persist in the lesion does not run parallel with the presence of such a substance in the blood as shown by this test. If Rosenthal and Raiga obtained better results in their cases by autohemotherapy it is hard to believe that this fact is due to any alteration of this substance in the blood. The failure to corroborate their results has induced us to study this phase of the problem more fully. The failure of the bacteriophage, recovered from the wound either after twenty-four or forty-eight hours, to act at incubator temperature would seem to indicate that it is less potent than the original bacteriophage, and it is, therefore, overwhelmed by the growth of the organisms at the temperature most suitable for their propagation. This temperature is, of course, the temperature at which bacteria grow in the body, and therefore, the inaction of the bacteriophage at body temperature may be of some significance.

The weakening of the bacteriophage by its habitation in the lesion for twenty-four hours was also indicated by the development of secondary growth after lysis. With the potent bacteriophages originally employed, secondary culture formation seldom occurred, but with the bacteriophages obtained from the lesion, either those arising spontaneously or those persisting for a day or two after instillation, secondary growth was the rule.

All of these results fail to support the idea that the bacteriophage propagates itself in the lesion. This points to the necessity of frequent reinoculations of the bacteriophage into the lesion. If bacteriophage is not reinjected into the lesion, at the end of twenty-four hours the growth of bacteria untouched by the original injection may continue unabated. These facts need further elucidation, but in the meantime there is a very definite indication for the frequently repeated administration of large quantities of bacteriophage, if it is to be used at all. Until we know what is inhibiting its propa-

gation in the body or in the lesion we must expect very little action following its injection at a distance from the lesion, except whatever may be gained from the injection of the lytic products of the bacteria.

In attempting to correlate the clinical results with certain laboratory findings, it would appear from the above observations that the presence or absence of antiphage in the blood as determined by Rosenthal's method has little or no effect on the usefulness of bacteriophage treatment. This point will be studied further and enlarged upon in another paper. However, the persistence of bacteriophage in the lesion and the persistence of susceptibility of the culture to the bacteriophage, as well as the absence of the development of progeny having cultural characteristics different from the original culture, are all factors of considerable importance. These three may all be important because they may indicate the potency of the bacteriophage. If there can be any other simple laboratory method of determining the potency of bacteriophage and maintaining its potency, the problem will be greatly simplified. But the efficiency of any treatment is determined to a great degree by the phase of the disease in which the treatment is begun. This is particularly true of infections for even after the infection is under control the process of healing is greatly modified by the kind and extent of the tissues which have been destroyed.

SUMMARY

One hundred and ten cases of furuncles and carbuncles have been studied from the point of view of the cultural characteristics of the strains of staphylococcus obtained from them and the susceptibility of those strains to the lytic action of bacteriophage as well as the changes in the cultural characteristics of these strains both in the test tube and in the lesion following the repeated administration of the bacteriophage. Certain features of the behavior of the bacteriophage and its persistence in the lesion have also been studied, and an attempt has been made to correlate the persistence of the bacteriophage or the disappearance of it in the lesion with the absence or presence of an inhibiting substance in the patient's serum known as antiphage. Our findings were briefly as follows:

1. A relationship could not be demonstrated between the hemolytic or nonhemolytic properties of the staphylococci and their susceptibility to bacteriophage. Of the 110 strains tested, only three were resistant. In the whole series, 81 were hemolytic *Staphylococcus aureus* and 18 were nonhemolytic *Staphylococcus aureus*. The rest were intermediate or mixed strains. These tests were all made with freshly isolated strains of bacteria and with several potent bacteriophages. The results indicate that if potent bacteriophages are employed practically all strains of staphylococci from acute lesions can be lysed.

2. The bacteria in the lesions were found to be altered in their cultural characteristics following the repeated injections of bacteriophage. After twenty-four hours the colonies on the blood plates showed degenerative changes, areas of absent growth, and "a moth-eaten appearance." At forty-eight hours these changes were not as marked, although after more injections, the colonies sometimes became roughened and pinpoint in size. A few

changed in their chromogenic properties and some in their hemolytic properties. Some became more resistant to bacteriophage and still others less resistant. In the majority of instances, the cultures remained unchanged in their susceptibility to bacteriophage. It was seldom demonstrated that the lesions became absolutely free of bacteria either after repeated injections of bacteriophage or when the bacteriophage was generated spontaneously in the lesion, although in two cases negative cultures were obtained both on the plate and in broth. This may have been due to inhibition by bacteriophage rather than actual destruction, for subsequent cultures were positive.

3. After one injection of bacteriophage, the lytic principle could be reisolated from the lesion at the end of twenty-four hours and forty-eight hours. At these intervals the bacteria showed evidence of destruction, particularly at the twenty-four-hour period. The bacteriophage could also be recovered from the filtrate of cultures incubated at room temperature after seventy-two and ninety-six hours, after instillation into the lesion, but in a much weakened state. While the bacteriophage persisted in the lesion and still had some destructive action after twenty-four hours, it did not increase in potency as it did in the test tube. Instead, it grew progressively weaker.

4. In about a third of these cases, the serum of the patients was tested for the presence of "antiphage," a substance which has the power to inhibit the action of bacteriophage in the test tube. This principle was found in the blood serum of a number of these patients, but there was no correlation between this fact and the persistence or disappearance of bacteriophage in the lesion.

5. The favorable clinical effect of the use of bacteriophage seems to have some correlation with the persistence of bacteriophage in the lesion and the maintenance of the cultural characteristics and susceptibility of the strain producing the disease and may have little or nothing to do with the presence or absence of the so-called "antiphage" (bacteriophage inhibiting substance) in the blood serum of the patient.

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CORONARY OCCLUSION AND SUDDEN DEATH

A Study Based Upon a Review of 345 Necropsies Performed at the Board of Health Laboratory, Ancon, Canal Zone, Between April 15, 1932, and April 15, 1933

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HISTORY

CORONARY occlusion was recognized and described at autopsy by pathologists forty to fifty years before wide medical interest was aroused in the disease about 1918. Coronary disease "has only been generally recognized and found to be extremely common since about 1926," according to Rolleston,[1] who also states that the condition was first described adequately by Weigert in 1880, but that a clinical diagnosis of coronary thrombosis was made in 1876 by Adam Hammer. In a general review, Benson[2] traces first observations on coronary sclerosis to Drelincourt in 1700. Dock recognized the disease during life in one patient, and in 1896 reported the case with autopsy confirmation.[3] In 1910, in Germany, two Russians, Obrastzow and Straschesko[4] adequately described the clinical syndrome. Herrick[5] soon described the disease in America. Osler[6] early correlated the clinical and pathologic findings; he believed that the disease was one which affected mainly the upper classes, and he used as examples, John Hunter, Charcot, Nothnagel, and William Pepper, each of whom died of coronary disease.

CASE REPORTS

There are a few general considerations to be mentioned before reviewing the cases listed in Table I. A constriction of 95 per cent or more of the arterial lumen is considered as occlusion in this paper. All autopsies were performed within twenty-four hours postmortem, and included examination of the brain, microscopic examination of all organs, and except in embalmed bodies, serologic and chemical examination of the blood and spinal fluid. In the embalmed bodies fixing fluid generally was flowing into the right common carotid artery within three hours after death. Cases 1 to 11, inclusive, are of coronary sclerosis; Cases 12 to 15, inclusive, are of coronary sclerosis with thrombosis; Cases 16 and 17 are of constriction of the coronary orifice by syphilitic aortitis; and Case 18 is of coronary embolism from a mitral valve vegetation.

DISCUSSION

Age.—As shown in Table II, the fifth and sixth decades claimed the most cases. This frequency is one decade younger than the usual published figures,

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and may be explained by the relatively younger ages of the inhabitants of the Zone. However, this is not a disease of the aged as shown by Conner and Holt,[7] who in a study of 287 cases found that initial symptoms occurred before fifty-one years of age in 33 per cent and before sixty-one years of age in 75 per cent. According to Eggleston[3] the disease is rare before forty, not uncommon between forty and fifty, and most frequent between fifty and seventy. The ages of the embolic and of the syphilitic constriction of the orifice cases may be much younger. The age of a black female who recently died suddenly from syphilitic atresia of the left coronary orifice was sixteen years.

Sex.—There were many more males (87.5 per cent) than females (12.5 per cent), and since in the Canal Zone all cases of sudden death are sent to the morgue for autopsy these figures probably represent the existing situation. Published series show a variation from about 10 to 30 per cent in the number of females affected.

Race.—Although in autopsies on 286 bodies over one year of age only 78 were white, the number of coronary sclerosis deaths was exactly twice as numerous in the white as in the black race. Considering all types of coronary occlusion there was a marked preponderance in the white race, the usual finding.

Symptoms.—The symptoms are best demonstrated by the four hospitalized cases (2, 8, 10 and 14), three of which showed sclerosis only and one of which showed sclerosis with thrombosis. Dyspnea occurred in all four cases. Cardiac pain, gastric symptoms (of nausea, vomiting, or epigastric discomfort), fever (because of the profuse sweating the oral temperature reading may be below normal, but a rectal temperature reading will generally indicate the presence of fever) and/or cough occurred in three. In two cases tachycardia, low blood pressure and leucocytosis were found. Enlarged heart, pericardial friction rub, and albuminuria were found once. The electrocardiogram was used once and correctly aided in the diagnosis. It is interesting to note that in a study of coronary occlusion at Peter Bent Brigham Hospital in 1923, Wearn[8] stated that there was "no typical or characteristic electrocardiographic curve of the condition." The clinical symptoms are due to the ischemic necrosis of the myocardium and they are well described in the literature,[9] and in recent textbooks. Hamman[10] has collected the symptoms into four groups based upon the pathologic changes, as follows: (1) The immediate symptoms associated with the lesion, the anginal seizure; namely, pain and shock manifested by prostration, fall in blood pressure, and suppression of urine; (2) the symptoms associated with the myocardial change; namely, dyspnea, feeble cardiac impulse, and chronic passive congestion manifested by cyanosis, pulmonary edema, enlarged liver, and albuminuria; (3) the symptoms associated with myocardial infarction; namely, fever and leucocytosis, pericarditis, and embolic phenomena; and (4) additional symptoms such as gastric, vasomotor and nervous.

The longest duration of symptoms before death in sclerosis was three and one-half years (Case 2), and then the patient died suddenly. One patient had symptoms for six months; 2 had symptoms for two months; and 2 had some previous but slight symptoms. In 3 the duration of symptoms is unknown. Since the mortality of the first attack is only about 16 per cent,[7] prompt recogni-

TABLE I

CASE	AUTOPSY NUMBER	AGE	SEX	COLOR	CLINICAL RECORD	HEART			OTHER ARTERIOSCLEROSIS	GENERALIZED PASSIVE CONGESTION	REMARKS
						WEIGHT	MYOFIBROSIS	ACUTE GROSS DEGENERATION			
1	9778	44	M	B	Several attacks of precordial pain and dyspnea last 6 mo. Sudden attack 3 days before death of pain, nausea, dyspnea, prostration.	740	+	+	+	+	Microscopic luetic aortitis.
2	9787	55	M	W	Epigastric pain, dyspnea, palpitation, hyperacidity called gastritis 3 years before death. Hospitalized 10 days for anorexia and gastric discomfort, suddenly fell dead. B.P. 103/54. Normal temperature.	335	+	+	+	+	Embalmed body.
3	9818	53	F	W	Hospitalized 4 yr. Involutional psychosis; hypertension. Fever and prostration last 2 days.	280	+	−	+	+	Albuminuria. Microscopic myocardial infarction.
4	9826	56	M	W	Sudden substernal discomfort at night; fell dead coming from bathroom.	535	−	+	+	−	Embalmed body. No head exam.
5	9845	40	M	W	Sudden epigastric and respiratory distress and death while fishing.	450	−	+	+	+	Embalmed body.
6	9921	59	M	B	Arose from sleep, went to bathroom, fell dead.	445	+	+	+	+	
7	9972	50	M	W	Sudden nausea and death following sexual excitement.	450	+	+	+	+	Embalmed body.
8	9994	72	M	B	In Leper Colony 12 yr. Acute abdominal pain 2 mo. before death with fever, cough, tachycardia, hemoptysis, albuminuria, anasarca.	690	+	+	+	+	Syphilitic aortitis. Wassermann four-plus.
9	10059	45	M	B	Sudden acute respiratory distress and substernal pain. Death within half hour. Similar but mild and transient attacks previously.	425	+	+	−	+	Coronary constriction of only 80%. Syphilitic aortitis.
10	10088	44	M	B	Sudden nausea and vomiting after lunch. B.P. 94/64; T. 96.8° F.; sweating; W.B.C., 10,000; polys. 78%. Death within 12 hr.	460	−	+	+	+	

TABLE I—CONT'D

CASE	AUTOPSY NUMBER	AGE	SEX	COLOR	CLINICAL RECORD	HEART			OTHER ARTERIOSCLEROSIS	GENERALIZED PASSIVE CONGESTION	REMARKS
						WEIGHT	MYOFIBROSIS	ACUTE GROSS DEGENERATION			
11	10108	57	M	W	Awakened by sudden heart pain; respiratory distress and death within half hour. No previous symptoms.	350	+	+	+	+	No albuminuria
12	9815	57	M	W	Suddenly fell dead at home.	385	+	+	+	+	Embalmed body
13	9926	63	M	W	Awakened by coughing spell. Dead within one hour. Previous moderate cough and fluttering heart.	395	+	+	+	+	Embalmed body
14	9988	46	M	W	Hospitalized 8 days before death with sudden precordial pain. Fever; tachycardia; B.P. 130/90; W.B.C. 7,400; polys. 71%; typical E.K.G. Pericardial friction rub 2 days before death. Cough.	450	+	+	+	+	Embalmed body. Endocardial thrombus. Fibrinous pericarditis. No head examination.
15	10100	62	M	W	Recently married. Died suddenly in bathroom. Slight indigestion for last 3 mo.	535	+	+	+	+	Cholelithiasis
16	9871	36	M	B	Sudden collapse and death during first hour's work in morning. No previous symptoms.	410	+	+	−	−	Atresia of anomalous coronary orifice by syphilitic aortitis.
17	9946	58	M	B	Collapsed while walking; death within an hour.	480	+	−	−	+	Microscopic myocardial infarction. Syphilitic aortitis. Wassermann fourplus.
18	9797	26	F	B	Four months endocarditis lenta. Sudden prostration 40 hr. before death; B.P. 90/65; sweating; respiratory distress.	570	−	+	−	+	Subacute bacterial endocarditis, mitral. Terminal pericarditis.

TABLE II

TYPES OF CORONARY OCCLUSION

	CORONARY SCLEROSIS WITHOUT THROMBOSIS	CORONARY SCLEROSIS WITH THROMBOSIS	SYPHILITIC ATRESIA OF CORONARY ORIFICE	CORONARY EMBOLISM	TOTALS
No. of Cases	11	4	2	1	18
Age 26	0	0	0	1	1
36	0	0	1	0	1
40-47	4	1	0	0	5
50-59	6	1	1	0	8
62-63	0	2	0	0	2
72	1	0	0	0	1
Sex					
Male	10	4	2	0	16
Female	1	0	0	1	2
Race					
White	6	4	0	0	10
Black	5	0	2	1	8

tion is an advantage for earlier and prolonged rest treatment and a better functional outlook.

Pathology.—Unfortunately "coronary thrombosis and infarction of the heart are generally spoken of in the same breath as if they were one and the same thing,"[12] whereas ischemic necrosis of the myocardium may result from only an extreme degree of narrowing of the coronary arteries, a lesion too seldom stressed in the literature. That "the obstruction is almost always due to the development of a thrombus"[3] is not borne out by postmortem findings (Boyd,[12] Willius,[13] and Bedford[14]). The clinical use of the term "Coronary Occlusion, due to . . . (type of lesion)" is suggested.

In every case of sclerosis with or without thrombosis the occlusion was in the left coronary artery, and in only one case was it not in the anterior descending branch. The typical location for the largest atheromatous patch was from 2 to 3 cm. beyond the bifurcation of the main artery. In coronary disease the statistical evidence is in favor of a diagnosis of left coronary occlusion unless there is some definite evidence for right coronary occlusion, such as emboli in the lungs from a mural thrombus in the right ventricle. The right coronary artery was the seat of the lesion only in the case of embolism. The left coronary was the seat of the lesion in 16 of Wearn's[8] 19 cases. Of 33 cases with autopsy, Willius[13] found the left coronary artery occluded in 29, the right in 2, and both in 2.

The areas of ischemic necrosis generally appeared in the anterior portion of the left ventricle near the apex; in four cases they also appeared in the left side of the interventricular septum. Although in 10 cases there were distinct gross acute infarcts, in only 3 of them were there conspicuous white infarcts. It is necessary to closely inspect the myocardium because "acute infarct formation may frequently be overlooked in the gross specimen."[14] Ischemic necrosis was evident in every case microscopically. Such a heart embalmed immediately after death presents an easily discernible infarct which is characterized by a pink area of softness (devoid of arterial supply) surrounded by firm, fixed myocardium; postmortem blood clotting which has occurred before embalming may

obstruct a coronary artery and the surrounding myocardium may simulate an antemortem infarct, but then the microscopic picture of swollen muscle cells with granulation, loss of striation, shrunken nuclei, leucocytic infiltration and of a bordering zone of hyperemia is absent. An infarct which extends to the endocardium offers an opportunity for the formation of a mural thrombus (only one, Case 14, in this series) and resulting emboli, or, the infarct may extend to the pericardium and bring about a pericarditis. An infarct may become thin, form a myocardial aneurysm, and finally rupture. Myofibrosis, evidence of previous anemia, was found in all cases except the one of embolic occlusion. Generalized arteriosclerosis was found in 14 cases; and in 2 cases syphilis may have been the etiology of the sclerosis. An enlarged liver (1,750 gm. or over) occurred in only 50 per cent, a low figure. Albuminuria was present in 66 per cent of the cases in which urinalysis was done. Extracardial thrombi were found in only 2 cases. Meakins[11] in 62 cases of coronary artery thrombosis found extracardial thrombi in 40 cases.

TABLE III

SUDDEN DEATH, ALL CAUSES

	CORONARY OCCLUSION	RUPTURED AORTIC ANEURYSM	MYOCARDITIS	PULMONARY EMBOLISM	UNDETERMINED	HEAT PROSTRATION	TOTALS
No. of Cases	13	4	3	2	2	1	25
Age 19	0	0	0	0	1	0	1
20-29	0	1	0	0	0	0	1
30-39	1	0	1	0	1	0	3
40-49	3	1	2	0	0	1	7
50-59	7	1	0	2	0	0	10
60-69	2	0	0	0	0	0	2
70-79	0	1	0	0	0	0	1
Sex							
Male	13	4	3	0	2	1	23
Female	0	0	0	2	0	0	2
Race							
White	8	2	1	0	0	0	11
Black	5	2	2	2	2	1	14

Sudden Death.—Excepting the accidental and traumatic deaths there were 25 sudden deaths from all causes. Table III shows the various causes with age, sex, and race figures. Cerebral hemorrhage is a clinical diagnosis commonly made at sudden death. Death from cerebral hemorrhage occurred in only 3 cases within twenty-four, twenty-three, and fourteen hours, respectively, after the strokes. "Of all sudden and unexpected deaths, apoplexy furnishes the fewest autopsies and inquests."[15] The largest single cause was coronary occlusion, which was more frequent than all other causes combined. It is interesting to note that of 11 cases of sudden death in the white race, 8 were of coronary occlusion. Table IV shows the various types of coronary artery occlusion which caused sudden death. Major McNabb,[16] in reviewing the 348 autopsies he performed in a year at the Canal Zone Board of Health Laboratory, found 14 cases of sudden death caused by coronary occlusion, and that "60 per cent of sudden deaths were a result of myocardial failure resulting from coronary lesions." In

a recent study of 198 patients brought in dead, Bedford[14] found "coronary artery disease, 80 cases, and atheroma of coronary arteries, 63 cases."

TABLE IV

SUDDEN DEATH FROM CORONARY OCCLUSION

	SCLEROSIS	THROMBOSIS	SYPHILITIC ATRESIA OF ORIFICE	TOTALS
No. of cases	8	3	2	13
Age				
30-39	0	0	1	1
40-49	3	0	0	3
50-59	5	1	1	7
60-69	0	2	0	2
70-79	0	0	0	0
Sex				
Male	8	3	2	13
Female	0	0	0	0
Race				
White	5	3	0	8
Black	3	0	2	5

Scott[17] has aptly said "when a man beyond forty and in his usual health is seized with a severe attack of substernal pain, accompanied by shock, circulatory collapse, and dyspnea, we may assume that something definite has happened in the heart. If the patient dies in a few minutes, we may be reasonably sure that he has had a circulatory accident, most likely coronary. Indeed, more than 90 per cent of all individuals dying suddenly . . . will show at autopsy a cardiac lesion, coronary disease . . . or rupture of the heart at the site of an old or recent infarct. In a few cases a ruptured aorta is found. Very rarely does cerebral hemorrhage kill in a few minutes. In spite of these well-established facts we still find such erroneous diagnoses as 'acute indigestion,' or 'ptomaine poisoning' given as the cause of sudden death."

SUMMARY

In a study of 345 consecutive autopsies, of which 286 were upon bodies over one year of age, representing 208 negroes and 78 Caucasians, and which were performed one calendar year, there are presented: 18 cases of coronary occlusion produced by sclerosis without thrombosis (11), sclerosis with thrombosis (4), syphilitic constriction of a coronary artery orifice (2), and embolic occlusion (1); a discussion of the symptoms and pathology of coronary occlusion; and a discussion of 25 cases of sudden death, 13 of which were caused by coronary occlusion.

It is suggested that the inaccurate general term "coronary thrombosis" be used only when an actual thrombus is present.

The following *working rule* is suggested: *Unless there are signs definitely indicating other disease in a white male over forty years of age who dies within three hours after the onset of either respiratory, cardiac, or gastric symptoms, the cause of death is coronary occlusion.*

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ALPHA DINITROPHENOL AND ITS INFLUENCE UPON METABOLISM*

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MY INTEREST in this chemical substance was aroused during the Great War. The reports of cases of acute and others of chronic intoxication; the circulatory disturbances in the former and the progressive loss of flesh, anemia, and sweating in the latter, and even sudden death among some of the acute manifestations, naturally invited attention in relation to the manufacture of munitions, especially during that period when part of this industry devolved upon the United States. When the pernicious potentialities of certain of the chemicals utilized in the making of explosives were clearly recognized, successful methods of safeguarding those in the industry were introduced. It was this factor probably which caused a lag in the attention originally given to the subject. However, during the past two years especially, numerous articles have appeared, most of them in the French journals, particularly by pharmacologists and physiologists. Most of the published work has dealt with the effects of nitrated naphthols, specifically dinitro-alpha-naphthol, often referred to for the sake of brevity as D. A. N. Of the two isomers, alpha and beta-naphthol, the nitrated alpha-naphthol has received the greater attention. These two are derived from coal tar, or are prepared synthetically by hydrating the hydrocarbon naphthalene. The phenol group consists of nitrated hydroxyl derivatives of benzene. Both the naphthol and phenol groups are toxic in certain doses. Both are thermogenic, also in appropriate doses, but the naphthol group is more selective in its thermogenic properties, for certain animals fail to develop fever, irrespective of the route by which the drug is administered.

Renewed and widespread interest has been stimulated by the recent article by Cutting, Mehrtens and Tainter,[1] and still more recently by the publications of Anderson, Reed and Emerson[2] and by Tainter, Stockton and Cutting.[3] Each of these reports dealt with alpha-dinitrophenol (1: 2: 4), and, as the Preliminary Report of the Council[4] stated, "this compound is relatively new both to pharmacology and to clinical medicine." To those who know something of the literature of this subject, and particularly if they have had some clinical experience with this chemical, the advice of the Council will be regarded as sound. "There are limitations to and possible dangers from the use of the drug clinically. It should be used only under strictly controlled conditions." Pertinent to this quotation, it may be stated that the dose should be accurate, based upon the weight of the patient, and converting metric into apothecary's weight may introduce a possible error. I seriously question

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whether any prescription receives the calm and dispassionate attention it deserves, in the delicatessen and lunch counter drug store. Perhaps the abolition of the Eighteenth Amendment may aid in restoring that which has come to be spoken of as the older type of drug store.

In an editorial comment[5] on dinitrophenol, the writer raises the question as to whether there are cumulative effects from small doses repeated over long periods. To this it may be said that the drug has been given over a period of months with no appreciable ill effects. In doses over 10 mg. per kilo it will produce profuse, drenching sweats, fever, and very high metabolic rates. In the doses generally employed, 3 to 5 mg. per kilo, there is rarely even a slight elevation of temperature as measured by the clinical thermometer, no increase in heart rate, sweating is not always marked, but the metabolic rate begins to rise soon after the drug is taken, but falls to the patient's normal again in about forty-eight hours. The increased metabolic rate is not maintained after stopping the drug, even in those to whom it has been given over long periods of time. There is no proof therefore, based upon the specific action of the drug, that cumulative effects occur. Eruptions have been reported and edema, especially from the sodium salt which is much more soluble than the acid. Whether other idiosyncrasies exist, or whether it will manifest any ill effects in the presence of infections or disturbed metabolic states, cannot be stated definitely at this time. Both from animal experimental work, using nitrated naphthols, and from the clinical use of alpha dinitrophenol, it is fairly evident that these compounds should not be used in diabetes or in any phase of hyperthyroidism. Owing to the rapidity with which the average obese individual passes "from hand to mouth" so to speak, the difficulty in controlling diets, even in the hospital ward, in the absence of a well-organized metabolic department, and the almost uniform rapidity with which dinitrophenol speeds up metabolic rate, the chief interest seems to center in the possibility of weight reduction without detailed and specific supervision of the dietary. With this in view therefore, I solicited the cooperation of the metabolic department of the Philadelphia General Hospital, under the directorship of Dr. Edw. S. Dillon, Dr. W. G. Karr the metabolic chemist of the department, and Mr. Theodore V. Letonoff, assistant metabolic chemist.

The patient, J. S., a male, aged fifty-six, a chauffeur, was admitted to my ward on July 18, 1933. He complained of swelling and soreness of his ankles, of pain in various parts of his trunk and limbs, and that his excessive weight and dyspnea prevented him from working. Decidedly short, his average weight had been 168 to 170 pounds for some years. Nineteen months prior to his admission to hospital, he had reached 200 pounds. This remained fairly constant until seven months ago, when he began to gain more rapidly and went up to 245 pounds. Unable to work, probably economic conditions brought about a reduction in weight, for he was 214 pounds when first seen. He was not only obese, but he had in addition a nodular, circumscribed lipomatosis on limbs, thorax, and abdomen. As is not unusual, many of these areas were tender, he was subject to neuralgic pain, tired easily, a syndrome characteristic of adiposis

dolorosa and not unusual in the nodular, circumscribed variety. This nodular distribution he first noted thirty years ago and gradually the tumefactions increased in number, a few having become quite large. His bowels were regular, his habits good. He denied the use of alcohol. No syphilitic history, Kahn negative. Family history of no importance. His past history was good, but for a postnasal catarrh for some years and moderate deafness, especially in the right ear, which followed a severe cold ten years ago. For some months he had a productive cough and an occasional night sweat.

Physical Examination.—Not of pituitary type. No abnormal distribution of hair. Teeth in poor condition. A little pyorrhea. Tonsils enlarged. Few râles at bases of lungs. Heart: 13 cm. from midline to left border. No murmur. Muscle tone suppressed and distant. Pulse rate 80. Blood pressure, 142 systolic; 90 diastolic. Abdomen: No gross pathology noted. Genitalia: normal. Eyes: Pupils reacted normally to light. Veins overfull. No pathologic findings on examination of fields. Skull: X-ray of bones of skull was negative. No evidence of intracranial pressure. Sella slightly larger in vertical diameter than normal. A narrow bridge of bone passed from the anterior to the posterior clinoid, but the clinoids were otherwise normal. One of the circumscribed, fatty masses was removed for a biopsy. Dr. R. P. Custer reported the finding of typical adult fat supported by the usual fibrous trabeculae.

Before any of the drug was given to him, he had a basal metabolism rate of plus 6 on one occasion, plus 8 on another. His blood counts and urine were normal. Blood cholesterol 270, and his calcium 12.6 and 10.3 at different times. Liver function by the bromsulphaline and by the Quick, benzoic acid methods were both normal. Without dietary restriction, limiting fluid to 1,500 c.c. daily, he lost four pounds in nine days, his weight now being 210 pounds on July 27, 1933. On Aug. 9, 1933, he was placed upon a diet of

Proteins	Fats	Carbohydrates	Calories
100	50	100	1,250

Fifteen days later, on Aug. 24, 1933, he weighed 205 pounds, a total loss of but 9 pounds in thirty-seven days, approximately ¼ of a pound a day. On Sept. 18, 1933, he was admitted to the men's metabolic ward and the metabolic study was begun. At this time he weighed 197 pounds, a total loss of 17 pounds in sixty-two days, a little over ⅓ of a pound a day since his admission to the hospital. Up to this date he had received no dinitrophenol and during this period his basal metabolism rate was plus 6 on July 27, 1933, and plus 8 on Aug. 24, 1933. On Sept. 18, 1933, his fasting blood sugar was 100; Urea N. 0.013; CO_2, 59 vol. per cent. For the next three days he was kept upon the following diet:

Proteins	Fats	Carbohydrates	Calories
100	50	100	1,250

Sept. 22, 1933, his diet was changed as follows:

Proteins	Fats	Carbohydrates	Calories
85	60	200	1,680

Sugar Tolerance

Fasting	83 mg.
½ hour	132 mg.
1 hour	134 mg.
2 hours	90 mg.
3 hours	61 mg.

TIME	B.S. MG.	URINE GM. N. PER HR.	R.Q.	B.M.R.	CAL. PER HR. BURNED FROM PROT.	FAT	CARB.	WEIGHT
Sept. 25, 1933	No breakfast or lunch, regular supper							
A.M. 7:30	92							
6 A.M.–9 A.M.		0.511						
8:50			0.78	+ 6	13	49	14	
9:15	0.45 gm. dinitrophenol							
11:15	93		0.75	+37	11	87	12	
9 A.M.–12 Noon		0.415	0.74	+37				
P.M. 1:15					11	87	6	
12 Noon–3 P.M.		0.424						
3:15	102		0.74	+44	11	92	8	
Sept. 26, 1933	No breakfast							
A.M. 8:20			0.73	+24	12	71	6	
6 A.M.–9 A.M.		0.447						
9:00	0.45 gm. dinitrophenol							
11:00			0.73	+52	13	89	7	
9 A.M.–12 Noon		0.492						
P.M. 1:00	Regular lunch and supper		0.69	+54	13	98	0	
Sept. 27, 1933								
A.M. 8:50			0.73	+27				
9:00	Regular breakfast plus 0.36 gm. dinitrophenol							
11:00	Regular lunch and supper		0.85	+72				
Sept. 28, 1933								194
A.M. 8:50	101		0.80	+24	13	51	25	
9:25	100 gm. glucose plus 0.45 gm. dinitrophenol							
10:25	180		0.81	+38	13	55	31	
11:25	118		0.93	+41	13	12	76	
12:25	74		0.91	+33	13	20	62	
9:25-12:25		0.491						
Oct. 1, 1933	Readmitted to Men's medical ward							
Oct. 3, 1933								
A.M. 8:45			0.76	+ 7				193
Oct. 7, 1933				+36				

It will be noted that from Sept. 18 to Oct. 3, 1933, a total of fifteen days, he lost an additional four pounds in weight, or approximately one-quarter pound per day. Following his return to the medical ward, he was not given dinitrophenol for a couple of days, hence his return to a basal metabolic rate of plus 7, with a respiratory quotient of 0.76. The dose of 0.45 gm. given at one time on September 25 and 26, the first two days of the observation, was equivalent to 5 mg. per kilo. The same dose was given on September 28, but on September 27 the dose was less, being but 4 mg. per kilo. Despite the smaller dose on this day, the basal metabolism rate rose to plus

72, very much higher than it had done on September 25 and 26, which were fasting periods. We interpreted this as being due to the specific dynamic action of the food, plus the elevation of rate due to the drug. It will be evident, therefore, that in the ambulatory individual on a full diet, even smaller doses should be employed. One woman who was on a full diet and ambulatory, except during the period of study of her rates, with a basal rate of minus 27, was given but 1 mg. per kilo. One hour later her rate was minus 22, and after another hour it was minus 13. In none of the various observations above cited was there any appreciable change in temperature and pulse or respiration. On another occasion, I intend to present other phases of this subject, but at this moment my chief concern is to advocate a small dose once a day of 3 mg. per kilo, or even better, but 2 mg. per kilo over a long period of time, to the obese individual who is not a diabetic, and is engaged in a daily routine, i.e., not bedridden. This I regard as perfectly safe. Weight will not be lost rapidly and many days may elapse after the drug is first given before the weight begins to fall, but it is fairly steady and continuous subsequently. In the case of J. S., the subject of this special study, as previously stated, there was a loss of but 17 pounds in sixty-two days before dinitrophenol was given. Under a strictly controlled diet, three partial fasting days being included in a total of seventeen days up to the day of his discharge from the hospital on October 12, his weight fell to 187 pounds, a further loss of 10 pounds, or at the rate of three-fifths of a pound per day. The metabolic study in the case of J. S. shows the time factor in the development and the extent to which the basal metabolism rate is raised on a dose of but 5 mg. per kilo. At the same time the respiratory quotient was slightly lowered on September 25. With but one meal on this day and no breakfast next morning, a further lowering of the respiratory quotient is what would be expected. This progressive drop in the respiratory quotient, during the first two days of the study, does not necessarily mean that the oxidation of fat is stimulated, though very suggestive. The low calory intake must also be considered. It seems equally evident that the extra oxidation caused by the drug was not accomplished at the expense of protein or carbohydrate. The progressive increase in calories of fat burned per hour on each of the first two days, when considered in the light of the other findings portrayed, makes it very probable that the very evident increase in oxidation is at the expense of the fat. A side light is shed on this feature in our obese cases of cardiac decompensation. Presumably the interface phenomena of fat and water in the edematous cardiac case, in some way affords a firmer binding of the two. The basal metabolic rate does not mount quite so high, and until the tissue fluids have been mobilized and in large part excreted, there is no appreciable loss of flesh and the loss is very gradual when it does occur. An attempt to reduce the weight of the obese individual with myocardial involvement is manifestly justifiable, but it remains in doubt as to whether it is advisable to attempt it by means of dinitrophenol.

The study of sugar tolerance after 100 gm. of glucose plus 5 mg. per kilo of the drug, indicates that sugar utilization during the first hour may be inhibited. At the end of the second hour the blood sugar is essentially normal,

and the respiratory quotient of 0.93 is what one would anticipate, even had no drug been given. In experimental work on animals with dinitro-alpha-naphthol, an early rise in the blood sugar has been noted and the liver and muscle glycogen are reduced.

It is of further interest to note that a single dose of 5 mg. per kilo, given during the fasting state, caused a rise in the rate of 31 per cent in less than two and one-half hours, and a total rise of 38 per cent in about six and one-half hours. Twenty-four hours after the administration of the drug, the rate remained 18 per cent above his basal rate of the previous day. On another occasion, two hours after a dose one mg. less per kilo, given with his regular breakfast, there was a rise of 45 per cent in his rate, but this was regarded as a summation effect. The rapid action of the drug upon heat production in the fasting state was very similar on the two days. On the first day this was 31 per cent and on the second day 28 per cent, each in a little over two hours. Oxidation was much less rapid and less pronounced when the drug was given with a glucose meal, than when it was given alone and in the fasting state.

COMMENT

1. A metabolic study of the effects of dinitrophenol has been reported.

2. The rapid increase and degree of oxidation have been shown, (a) in the fasting condition, (b) when given with a glucose meal, (c) the summation effect of drug and regular meal.

3. The probability that oxidation occurs at the expense of fats has been suggested.

4. The question of dosage has been considered and in the light of present knowledge, doses of 2 to 3 mg. per kilo at one dose daily have been advised.

5. It would seem unwise to use it in a case of diabetes or in an edematous individual.

6. In appropriate doses, though its oxidative properties are striking, weight loss is not rapid, nor is there any appreciable effect upon temperature, pulse, or respiration.

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ABSORPTION OF DEXTROSE FROM THE HUMAN GASTROINTESTINAL TRACT*

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IN 1925, Cori,[1] as a result of an ingenious method by which absorption of saccharides from the entire gastrointestinal tract of rats was studied, came to the conclusion that (1) a proportionality exists between the absorbing surface of intestine and body weight in growing male and female rats, and (2) the rate of absorption of hexoses is constant and is independent of the absolute amount and of the concentration of sugar present in the intestine. He calculated the absorption rate to be 1.78 grams of dextrose per kilogram of rat.

Trimble, Carey and Maddock,[2] utilizing Cori's technic in dogs, made substantially the same observations. They found that the rate of absorption of dextrose from the intestinal tract of dogs is one gram per kilogram of body weight per hour; also, that this rate is constant and not influenced by: (a) the concentrations; (b) the absolute weight of dextrose ingested; (c) lengths of absorption period; (d) weights of the animals used; and, (e) excitement occasioned by experimentation.

MacKay and Bergman,[3] also employing Cori's method of study, made findings entirely at variance with those of the latter. They concluded that (1) the absorption rate of dextrose per unit of time from the intestinal tract of male and female rats of various ages bears a more constant relation to body surface than to body weight, and (2) the absorption coefficient (amount of dextrose absorbed per unit of body surface per hour) is raised by increases in either the amount or concentration of dextrose administered. Whatever the dose, the rate of absorption decreases with time after it is given. Pierce, Osgood and Polansky[4] and Burget, Moore and Lloyd[5] previously had made observations which are in agreement with those of MacKay and Bergman. In addition they found that the total amount of dextrose absorbed and the amount absorbed per 100 gram of body weight per hour varies within wide limits. These variations are not dependent on body weight or upon the actual amount of dextrose fed.

In animal experiments, then, the findings and conclusions of the various investigations are diametrically opposed to each other.

Jacobsen[6] found that the administration of water does not influence the "fasting" blood sugar level. Strouse[7] made similar observations, and in

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addition noted that the "fasting" blood sugar level is not changed by increased excretion of water. Boe[8] found in dogs that the administration of a dilute solution of dextrose causes only an insignificant rise in blood sugar; whereas a more concentrated solution caused a much higher rise. This may be taken as evidence that the more concentrated the solution the greater is the rate of absorption.

Direct methods of study of the absorption of dextrose from the gastrointestinal tract are, of course, not applicable to man. We are, as a consequence, deplorably ignorant of this subject as far as human beings are concerned. From a study of the respiratory quotient curve after the administration of dextrose, Johansson[9] concluded that the maximum rate of absorption of dextrose in man is between 80 and 100 gm. per hour.

In this paper will be reported the results of an investigation, by an indirect method, of the laws governing the absorption of dextrose from the human gastrointestinal tract.

In a normal individual it is practically impossible to raise the blood sugar level above 180 mg. per 100 c.c. of venous blood on the administration of dextrose by mouth, for the amount of insulin secreted is easily capable of causing the abstraction of dextrose from the circulation regardless of the rapidity with which it is absorbed from the intestine. In diabetes mellitus, however, hypoinsulinism exists and the amount of insulin secreted at any particular time is fixed and dependent on the severity of the case. The amount of dextrose that the individual diabetic can metabolize per unit time follows the equation determined by Allan.* [10] If the absorption of a certain amount of dextrose per unit time in a given diabetic gives rise to a certain blood sugar curve, the absorption of a greater amount will cause a higher blood sugar curve because the amount of insulin secreted remains constant. In the same diabetic, the greater the amount of dextrose absorbed in a given time, the higher will the blood sugar curve be. If, then, one gives to the same diabetic varying amounts of dextrose, two days apart, the blood sugar curves will be equal in height or higher with the larger dose, depending on whether the rate of absorption is the same or greater with the larger dose.†

METHOD OF PROCEDURE

With this a basis, experiments were performed on 14 female diabetics. To each one two doses of dextrose were given; a large one on one day, and a smaller one two days later. To a group of three, 125 gm. of dextrose were given on the first day and 25 gm. two days later; to a second group of six,

*The dextrose equivalent of insulin in any given case is not a constant, but is represented by the equation $g(u)^{0.85} = 10^{1.86}$ where g is the amount of dextrose metabolized by each unit, and u is the number of units of insulin given. The dextrose equivalent of insulin is increased if the amount of insulin remains constant and the amount of dextrose increased, or if the amount of dextrose remains constant and the amount of insulin decreased; the dextrose equivalent is decreased if the amount of insulin is increased and the amount of dextrose remains constant, or if the amount of dextrose is decreased and the amount of insulin remains constant. In this situation the amount of insulin remains constant. If more dextrose is absorbed the dextrose equivalent will be increased, so that the *rate* of rise of the blood sugar curve will be less and less with increasing rate of absorption of dextrose.

†It is realized that the blood sugar level is influenced by the secretions of the thyroid, adrenal and pituitary glands, and other factors. It is fair to assume that these factors, like insulin, remain constants during the period of experimentation.

either 100 or 150 gm. were given on the first day and 50 gm. two days later; to a third group of five, 150 gm. were given on the first day and 75 gm. two days later. The anhydrous dextrose was dissolved in distilled water and made up to 500 c.c. An hour after the administration of the dextrose 250 c.c. of water were given. The total volume was therefore 750 c.c. in each case for both the large and the small dose.

The dextrose was given in the morning after a fast of fourteen hours. Blood (venous) specimens were taken at the "fasting level," immediately before ingestion, and forty-five minutes, two hours, and in some cases, three hours after the ingestion of the dextrose. The sugar content of the blood was determined by the macro Folin-Wu method. The urine was collected at the end of the first, second, and in some of the cases, third hour after ingestion of the dextrose, and its sugar content determined by Benedict's method.

ANALYSIS OF RESULTS

The results are incorporated in Table I. After careful consideration it was decided that the best method of determining the influence of the different doses on the blood sugar curve was to calculate the differences in rise of blood sugar concentration when the large dose and the small one were given, from the "fasting level" to the "forty-five-minute," "two-hour," and "three-hour" levels, respectively. To illustrate: in Case 1 (Table I) the "fasting level" when 125 gm. of dextrose were given was 214, and the "forty-five-minute level," 332, indicating a rise in blood sugar concentration of 118 mg. per 100 c.c. of blood. When 25 gm. of dextrose were given the "fasting level" was 230 and the "forty-five-minute level," 332, indicating a rise in blood sugar concentration of 102 mg. per 100 c.c. of blood. The difference in elevations is 16. A plus sign is arbitrarily placed in front of the 16 to indicate that the rise is greater when the larger dose is given. A minus sign, on the other hand, indicates that the rise is greater when the smaller dose is given.

Analyzing the blood sugar concentrations at the "two-hour level" it will be observed that in Group I, in all cases, the rise is markedly greater with the larger dose. This also holds true for all the cases in Group II, although the difference is much less and in one case (Case 9) is within the limit of error of the method used to analyze the blood. In Group III, however, a striking change has taken place. Here, in four out of five cases, the rise is greater when the smaller dose is given. This is of fundamental significance for it indicates that at the end of two hours at least as much dextrose is being absorbed when 75 gm. are given as when 150 gm. are given; in other words, *the rate of absorption of dextrose is the same regardless of the absolute amount in the intestine.* As a corollary to this it may be stated *that the rate of absorption is constant.*

An interpretation may now be given for the greater rise in blood sugar concentration at the "two-hour level" in Groups I and II when the larger dose is given. The reason is that the dextrose, when the smaller dose is given, has been completely absorbed before the end of two hours so that the blood sugar concentration has been dropping for some time before the "two-hour

level'' is reached. This is brought out in Group II at the ''three-hour level.'' Here in every one of the four cases studied, the difference in rise is decidedly greater than at the ''two-hour level.'' Also, in Group I, the difference in rise at the ''two-hour level'' is much greater than in Group II because the blood sugar concentration in the former has been dropping for a much longer time.

It follows, then, that when 75 gm. of dextrose are given (Group III), the amount remaining in the gastrointestinal tract at the end of two hours is less than 25 gm.; whereas when 150 gm. are given, the amount remaining at the end of two hours is between 75 and 100 gm. The important deduction from these findings is *that the amount of dextrose absorbed in two hours from the gastrointestinal tract of the average adult diabetic lies somewhere between 50 and 75 gm.* Since there is no reason to suppose that the rate of absorption differs in normals from diabetics, this statements holds for all adults.

Analyzing the ''forty-five-minute level'' one finds that in 13 out of the 14 cases the rise is greater with the larger dose. In 6 out of the 14 cases the figure for the difference in rise is within the limit of error of the method used for analyzing the blood. In spite of this, one is impressed that the difference in rise is somewhat greater when the larger dose is given. It is no greater in the group receiving 25 gm. than in the one receiving 75 gm. What is the cause for this greater rise with the larger dose at the ''forty-five-minute level''?

It was stated above that some investigators[3, 4, 5] found that the rate of absorption is increased by increase in concentration of dextrose administered; also, that Boe[8] observed that the level of the blood sugar curve is higher when a more concentrated solution is given. In the experiments recorded in Table I, the volume was kept constant; the concentration necessarily varied.

The question then is: Is the greater rise with the larger dose due to the fact that the solution administered was more concentrated? To determine this point, the following experiment was performed. To seven patients, suffering from diabetes mellitus, varying doses of dextrose, of equal concentration, namely 32 per cent, were given two days apart. On one day 125 gm. and two days later 45 gm. of dextrose were administered. Venous blood was taken at the ''fasting level'' immediately before ingestion, and forty-five minutes after ingestion. The results are incorporated in Table II. It is to be observed that in 6 out of 7 cases the rise is greater with the larger dose, and that the differences in rise are of the same magnitude as in Table I. The conclusion to be drawn is that the height of the blood sugar curve is not influenced by differences in concentration of the dextrose administered. As a corollary it may be stated that the rate of absorption of dextrose is not influenced by this factor. I am at a loss to explain the greater rise in blood sugar concentration with the larger dose at the ''forty-five-minute level.''

The observations made above have a practical value. The dextrose tolerance test, especially when combined with a simultaneous study of the respiratory quotient curve,[11] constitutes the best method of determining the presence of diabetes mellitus and also its severity.[12] In a symposium on the

dextrose tolerance test, Gray[13] lists the test substances and doses used by various investigators in the study of carbohydrate metabolism. They are as follows: from 20 to 25 gm. of dextrose; 50 gm. of dextrose; 70 gm. of dextrose; 100 gm. of dextrose; from 150 to 200 gm. of dextrose; 100 gm. of levulose; from 50 to 100 gm. of cane sugar; 25 gm. of starch; 50 gm. of starch; from 70 to 100 gm. of starch; and a mixed meal. In addition it may be mentioned that Janney and Isaacson[14] use 1.75 gm. of dextrose per kilogram of body weight. The choice of these doses and substances in all instances is purely arbitrary.

John[15] performed dextrose tolerance tests on 50 patients with diabetes, using 100 gm. of dextrose as a test dose. He found that in 86 per cent of the cases the maximum rise occurs within two hours after the ingestion of the dextrose. This also holds true when 150 gm. of dextrose are given, for in 5 cases in which this dose was given by me (see Table I) all had the maximum rise within the first two hours. The character of the curve *after*

TABLE I

REPEATED DEXTROSE TOLERANCE TESTS IN DIABETICS USING VARYING AMOUNTS OF DEXTROSE (VOLUME OF FLUID CONSTANT)

	CASE NO.	DOSE OF DEXTROSE IN GRAMS	CONCENTRATION OF SUGAR IN BLOOD*							EXCRETION OF DEXTROSE IN URINE AFTER INGESTION IN GRAMS		
			FASTING LEVEL	FORTY-FIVE MINUTES POST CIBUM	DIFFERENCE IN RISE	TWO HOURS POST CIBUM	DIFFERENCE IN RISE	THREE HOURS POST CIBUM	DIFFERENCE IN RISE	FIRST HOUR	SECOND HOUR	THIRD HOUR
GROUP I	1	125	214	332	+16	500	+216			0.38	3.57	
		25	230	332		300				0.14	0.77	
	2	125	150	250	+33	300	+ 83			0.35	4.17	
		25	187	250		250				0.20	0.24	
	3	125	230	374	+70	460	+230			2.14	3.25	
		25	300	374		300				1.35	0.43	
GROUP II	4	100	214	374	+ 8	460	+144			0.62	6.34	8.31
		50	258	410		360				2.0	7.96	6.00
	5	100	300	416	+20	500	+104	428	+134	8.73	16.30	7.80
		50	278	374		374		272		5.25	8.48	3.50
	6	100	230	322	- 4	360	+ 34	372	+110	2.10	9.90	4.24
		50	278	374		374		260		2.57	5.30	1.28
	7	100	375	554	+ 8	600	+ 54	556	+128	7.00	18.50	12.80
		50	375	546		546		428		7.26	19.33	2.80
	8	150	263	468	+71	536	+157	500	+209	2.68	11.80	12.3
		50	312	446		428		340		3.50	7.90	2.62
	9	150	192	332	+12	332	+ 12	262		1.80	5.90	4.00
		50	172	300		300				1.20	3.90	
GROUP III	10	150	246	428	+36	428	-106			0.60	1.20	
		75	258	404		546				1.20	3.60	
	11	150	227	400	+13	484	+ 43	374		2.00	5.66	7.70
		75	214	374		428				0.90	4.00	
	12	150	166	300	+34	300	- 68	240		1.42	4.84	
		75	172	272		374				0.60	1.00	
	13	150	192	352	+54	322	- 40	250		3.50	8.90	4.75
		75	178	284		348						
	14	150	333	546	+45	600	- 33			3.94	10.50	
		75	300	468		600				3.25	7.10	

*In milligrams per 100 c.c. of blood.

the first two hours is determined by the size of the dose; the larger the dose, the longer it will take for the "fasting level" to be attained. In performing a dextrose tolerance test it is of value not only to determine whether the blood sugar curve is or is not higher than normal but also the maximum rise obtainable. From Table I it can be seen that 75 gm. of dextrose is the smallest dose that will give a maximum rise in blood sugar in the average adult diabetic.

It is also important to note that a dose of 50 gm. of dextrose or less may fail to disclose a potential or very mild diabetic if the "fasting level" is normal. The reason is that because of the complete absorption of the dextrose before the end of two hours post cibum, the blood sugar may drop to the "fasting level" at the end of the two hours. This event would not occur if 75 gm. of dextrose are given. Seventy-five grams of dextrose is the minimum dose that can be used to detect diabetes mellitus. It is accordingly proposed that 75 gm. of dextrose be used as the test dose.

A point which is not entirely relevant to the subject of this paper may, however, with value be discussed here. Does the administration of large quantities of dextrose, such as the patients received in these experiments, lead to a breaking down of tolerance? Joslin[16] strongly deprecates the use of the dextrose tolerance tests in diabetics, although he has never seen one injured by them. Ohler[17] also argues that on theoretical grounds the giving of large quantities of dextrose is harmful. Allen,[18] in his work on partially depancreatized dogs, found that excessive feeding of carbohydrates, especially where given in a rapidly absorptive form, such as dextrose, leads to a rapid breaking down of tolerance. Several years ago I[12] observed for several months seven diabetics who had had two dextrose tolerance tests performed two days apart. The test dose was 100 gm. of dextrose. None showed the slightest change in tolerance as a result of this procedure. Nor was there any depreciation of tolerance in any of ten patients of Table I as a result of receiving

TABLE II

REPEATED DEXTROSE TOLERANCE TESTS IN DIABETICS USING VARYING AMOUNTS OF DEXTROSE (CONCENTRATION OF SOLUTION CONSTANT)

CASE	DOSE OF DEXTROSE IN GRAMS	CONCENTRATION OF SUGAR IN BLOOD*		
		FASTING LEVEL	FORTY-FIVE MINUTES POST CIBUM	DIFFERENCE IN RISE
1	125	258	428	+19
	45	223	374	
2	125	214	374	+22
	45	246	374	
3	125	142	200	+12
	45	130	176	
4	125	131	196	+10
	45	107	162	
5	125	283	428	+29
	45	258	374	
6	125	227	374	+52
	45	205	300	
7	125	250	384	− 4
	45	238	376	

*In milligrams per 100 c.c. of blood.

the large quantities of dextrose. It may be stated categorically that the giving of large quantities of dextrose does not cause the slightest depreciation of carbohydrate tolerance in diabetics.

SUMMARY AND CONCLUSIONS

The absorption of dextrose from the gastrointestinal tract has been studied quite intensively in animals. The results and conclusions of the various investigators are conflicting.

This subject has been almost completely neglected in man.

An indirect method of studying this subject in human beings is described. It consists in performing repeated dextrose tolerance tests on diabetics, using varying doses of dextrose. The rationale for this procedure is outlined.

The conclusions drawn from the data obtained are as follows:

1. The rate of absorption of dextrose from the human gastrointestinal tract is the same regardless of the absolute amount present. A corollary to this is that the rate of absorption of dextrose is constant.

2. The amount of dextrose absorbed from the gastrointestinal tract of the average adult in two hours lies somewhere between 50 and 75 gm.

3. The rate of absorption from the gastrointestinal tract is not influenced by the concentration of the solution of dextrose administered.

4. Seventy-five grams of dextrose is proposed as a standard dose to be used in performing a dextrose tolerance test.

5. The ingestion of large quantities of dextrose by patients with diabetes does not cause the slightest depreciation of their tolerance for carbohydrates.

NOTE: I wish to express my thanks to Miss Louise Zelenko for technical assistance.

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HISTOCHEMICAL STUDIES OF ORGANS OF TUMOR-BEARING RATS BY THE MICROINCINERATION METHOD*

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THE study of the amount, character and distribution of mineral matter in normal and malignant tissues has been recently advanced by the introduction of the microincineration method (Policard,[1] Schultz-Brauns,[2] Scott and Horning[3] and others). The value of the observations made, especially those of comparative character, is, however, impaired by the fact, that the great majority of the investigators used paraffin sections in their work.

Fixation and dehydration of tissues cause not inconsiderable changes in the amount and distribution of the mineral substances in the cells (Schultz-Brauns,[2] Policard and Okkels,[4] Tschopp,[5] Cowdry[6]). The degree and type of changes produced by fixating and dehydrating agents depend upon their chemical nature, a time factor and possibly also on the physicochemical constitution of the tissues.

Many of these defects in the proper preparation of sections for microincineration have been eliminated in the technic of frozen sectioning developed by Schultz-Brauns,[2] who modified the commonly used method by cutting unfixed tissue with a knife cooled with carbon dioxide to –15° to 20° C. Losses of mineral substances are thereby completely avoided and the possibility of artificial displacements of the cellular mineral skeleton is markedly decreased.

The present communication represents a report of the results of an investigation on the mineral skeleton of malignant cells studied in frozen sections of fresh, unfixed tissue cut according to a modification of the method of Schultz-Brauns (Hueper[7]). In the second part of the paper observations are recorded which were made on normal and neoplastic tissues of rats treated with parathormone and viosterol.

PART I. MINERAL STRUCTURE OF MALIGNANT TISSUE

The tumor tissue used was obtained from rats which had been inoculated with a transplantable sarcoma, Philadelphia No. 1 and a transplantable medullary carcinoma, Walker No. 256 respectively, a detailed histologic description of which has been published in a previous paper (Waldschmidt-Leitz, McDonald, Hueper and Coworkers[8]). Sections were prepared with the cooled

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knife technic from fresh, unfixed tumor tissue removed from the peripheral parts of the tumors. Alternating sections were stained and incinerated respectively. For the incineration an electrically heated quartz furnace was used, the maximum temperature of which was adjusted to 600° C. The sections were kept in the oven for about forty-five minutes. They were left in the incinerator until they were cool and were then covered with a glass coverslip which was sealed with paraffin. A Zeiss cardioid condenser was used for the dark-field examination. A small Leitz camera which had no bellows and which could be attached to the eyepiece was used in the photographic work. The sections to be stained were first put into 95 per cent alcohol and then stained in the ordinary way with hematoxylin-eosin or hematoxylin-phloxin.

The stained sections show more or less densely packed tumor cells, embedded in a loose connective tissue stroma. The nuclei are relatively large and contain dark stained chromatin material arranged in irregular lobules and threads. A complete or incomplete ringlike accumulation of the chromatin in the periphery of the nuclei is exceptional. The cytoplasm is moderate to scanty in amount and is indistinctly outlined in the sarcomatous tumor, while rather well defined in the carcinomatous neoplasm. Small localized foci of degeneration and necrosis are scattered in moderate number in the tissue. In these degenerative areas pyknotic nuclei are numerous. Pseudopapillary-cystic formations are seen in some of the regressive foci. The necrotic areas are pink in color and contain chromatic nuclear fragments. Diffuse, usually perivascular hemorrhages are occasionally observed.

The ash pictures of the tumor sections vary considerably in different portions of the same section. In the areas corresponding to the above described healthy parts of the tumor a scanty bluish to bluish gray reflecting matrix of very small granules is present. Embedded in it numerous, smaller and larger, somewhat ill-defined, irregularly round shaped rings are seen. They are either exclusively or mainly composed of accumulations of fine, bluish reflecting particles. The center of these rings is sometimes empty, usually, however, filled by a small clump of the same bluish reflecting substance or of a mixture of the bluish and a coarse white reflecting matter. Small white reflecting particles occur also in some of the bluish rings. Reddish or brownish red reflecting granules are usually absent in the ring formations. They appear in only a few of the sections, where they participate in appreciable amounts in the composition of the rings and their central content in some areas. Surrounding the individual rings an indistinctly outlined area of less densely packed bluish granules fades away into the above described matrix.

The rings are much more distinct in those areas which show in the stained sections evidence of cell degeneration. They stand out as more or less solid, white reflecting formations, consisting either of a continuous or a discontinued, bead-like accumulation of coarse, white particles. The central portions of the rings are sometimes empty, but in general they are filled with the same white reflecting material of which the rings are composed. Solid

white, round bodies are often seen in such areas in place of ring formations and are apparently the result of a merger of the ring and its central content. White granules are also found in varying number in the zone surrounding the individual rings. There is not infrequently a second, outer ring present, consisting of white particles arranged in a bead-like formation which seems to demarcate the cell from the intercellular matrix. A larger amount of mineral matter, in which the white reflecting particles are prominent, is also found in the matrix.

The necrotic foci of the tumor tissue are characterized in the incinerated section by an incomplete or complete loss of the cellular structure. There are more or less dense, diffuse accumulations of white reflecting matter, which contains sometimes smaller and larger reddish brown particles.

As the interpretation and significance of the observations recorded depends to a certain extent upon the chemical nature of the various elements visible in the ash pictures, brief reference may be made to the information available at the present time on this subject. It is conceded by all investigators that the white reflecting particles represent calcium, probably in the form of calcium oxide. The brownish red to red granules seen in the ash are iron oxide. The nature of the bluish reflecting material is not definitely known. Policard suggested that it represents potassium and sodium. Scheid[9] has called attention to the presence of brownish, homogeneous, glassy or granular substances, which appear especially often in tissues containing large amounts of phosphatids, such as the white substance of the brain. These droplets, which may occasionally express a similarly colored liquid forming a network in the ash (artefact), are according to the investigations of Tschopp[5] and Scheid[9] phosphorus oxide. It may be mentioned in this connection that identical formations of droplet or network type have been observed in the incinerated sections of the two tumors examined and were found in general in close relation to degenerative and necrotic foci, where, however, also brownish red granules of iron character were frequently met.

COMMENT

The examination of the incinerated sections of the two transplantable rat cancers showed that the inorganic remains of the healthy tumor tissue are mainly bluish reflecting granules, which represent according to Policard's opinion sodium and potassium. Similar observations on the color of the ash of malignant and normal proliferating tissue were made by Olch[10] who reported that the ash of human breast and skin carcinomas and of the epithelial proliferations in chronic cystic mastitis, in hyperkeratoses and warts has a bluish color. Calcium appears either to be absent or to be present only in small amounts in the mineral skeleton of the healthy tumor cell. Iron does not seem to play any important rôle in the mineral constitution of the cytoplasm and the nucleus of the cells of the two tumors examined.

While the ring-like accumulations of mineral matter are located in the region where the nucleus is found in the stained sections, it appears to be doubtful, judging from the size and diameter of some of the rings, whether

these formations are located in the peripheral parts of the nucleus or are mineral matter absorbed to the nuclear surface or represent to a certain extent accumulations of minerals in the perinuclear space. These mineral rings can, however, not be looked upon as artefacts resulting from the action of fixating and dehydrating agents upon the cells, as suggested by some investigators.

These findings do not substantiate in some respects those recently made by Scott[3] and Scott and Horning,[3] who reported that malignant cells are characterized by an extraordinary concentration of iron and to some extent also of calcium in the peripheral margins of the nuclei resembling in this regard the mineral structure of embryonic cells. An explanation for this discrepancy cannot be offered at the present time. The only conclusion which can be justly drawn from the evidence at hand seems to be, that not all malignant cells resemble in their mineral structure that present in embryonic cells.

The observations made do not confirm another claim of Scott in regard to the presence of correlations existing between the mineral structure of the nucleus of malignant cells, the distribution of chromatin substance and the absorption picture obtained by ultraviolet ray photography. Scott has to a certain extent disproved his own contention in his experiments with opalinids, where he noted the absence of a relation between the scanty amount of nuclear ash and the good amount of chromatin present. The chromatin substance of nuclei of malignant cells in tumors in men, mice or rats is moreover not accumulated in the peripheral parts of the nuclei as contended by Scott. An accumulation of chromatin matter in this region is in general considered as evidence of cell degeneration. The location of the mineral substance as demonstrated by the incineration method does, therefore, not correspond to that of the chromatin matter of healthy tumor cells. The actual existence of the correlations cited above seems to be insufficiently supported by reliable experimental evidence.

In regard to the absolute amount of mineral matter in malignant cells, the investigations reported above do not support the conception that malignant cells have a higher mineral content than normal cells (Scott). This impression is gained by a comparison of ash pictures of areas of healthy tumor tissue with those of organs of similar cellularity, such as liver, spleen, pancreas, kidney, lymph node, salivary gland, etc. Much apparently depends upon the relative size of the cells and their functional state. Olch[10] reported that the cells of the resting mammary gland contain large amounts of calcium. Policard and Pillet[11] stated that proliferating tissue has a low ash content and Policard and Doubrow[12] noted that the ash content decreases with the increase in the embryonicity of the tumor. Schultz-Brauns mentioned during a brief discussion of the ash content of various tissues that benign as well as malignant neoplasms show in general a light to moderate amount of ash. He added, however, that marked variations may occur among the ash content of individual tumors.

Considerable differences in the density of the mineral ash exist also between different parts of the same tumor. These have been referred to variations in the vital status of the cells. The ash and especially its calcium com-

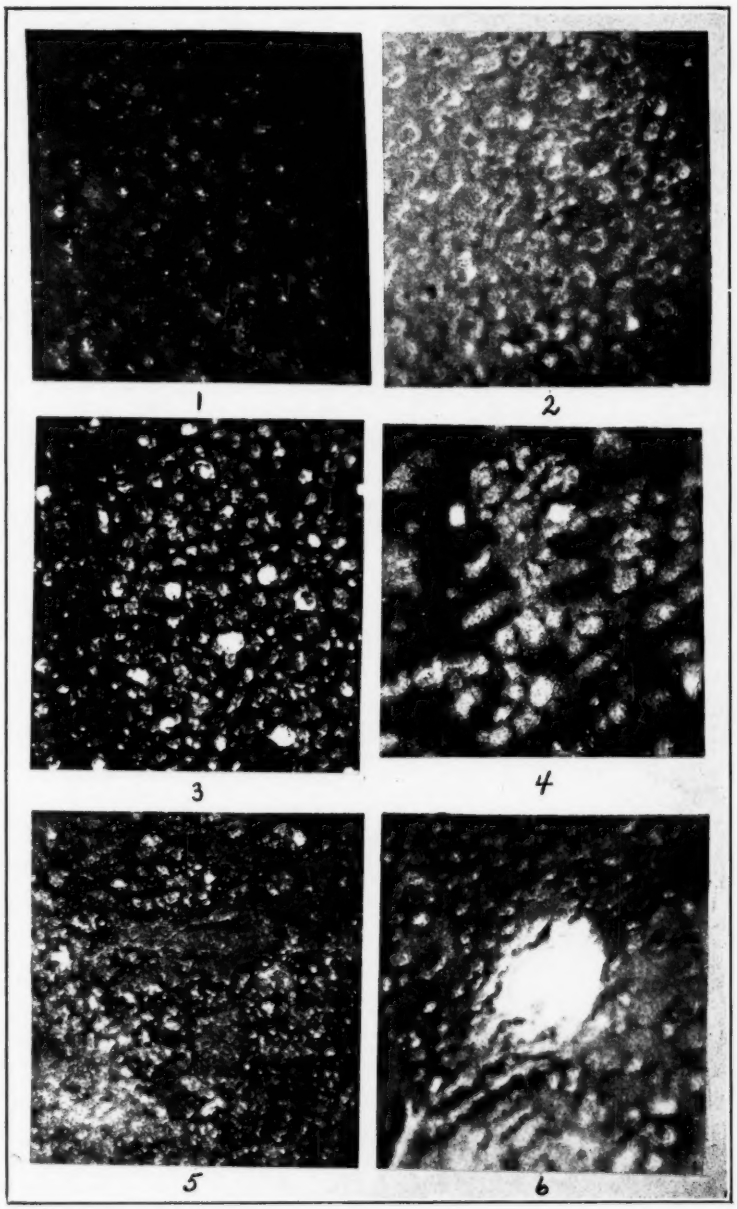

Fig. 1.—Incinerated healthy sarcomatous tissue showing rather faintly visible rings of bluish reflecting granules scantily mixed with white particles.

Fig. 2.—Ashed section of degenerating sarcomatous tissue with prominent white rings.

Fig. 3.—Degenerating sarcomatous tissue with white rings and central "calcifications."

Fig. 4.—Very edematous degenerating sarcomatous tissue with marked central "calcifications" and distinct white outer rings, demarcating the cellular outline.

Fig. 5.—Advanced degeneration of sarcomatous tissue with partial loss of cellular structure in the ashed section.

Fig. 6.—Necrotic focus in sarcomatous tissue characterized by dense accumulation of calcium particles.

ponent increases in direct proportion to the degenerative state of the cells and is apparently most abundant in areas of complete necrosis.

Shear[13] stated in his recent review on the rôle of different minerals (sodium, potassium, magnesium and calcium) in cancer, that it is the current view that the calcium content of tumors increases with increasing age and with increasing extent of necrosis. Policard and Doubrow[12] noted that degenerating and necrotic tumor tissue contains more ash than living tissue. Scott and Horning[3] remarked that their investigations furnished supplementary evidence for this observation. The experimental data obtained in this study confirm this conception and supply at the same time additional information in regard to the intracellular mineral changes and shifts which are associated with the process of degeneration.

An increase in the amount of calcium particles in the nuclear mineral ring represents apparently the first sign of a decreasing vitality and beginning degeneration of the cell. Following this nuclear calcinosis calcium particles are accumulated in increasing amounts in the central portions of the ring, until ultimately a union between the ash of the ring and of the central nuclear mineral deposits occurs. Accompanying this nuclear change there is also an increase in the number of calcium particles in the cytoplasmic region combined with a deposition of larger calcium granules in the peripheral parts of the cell (membrane?), where thereby a second outer ring is formed. With the ultimate complete disintegration of the cellular structure with progressing necrosis a definite mineral skeleton of the cells disappears and diffuse accumulations of mineral matter mainly representing calcium take their place. Older necroses are characterized by denser, almost solid masses of coarse calcium particles.

It was noted that necroses which showed in the incinerated sections marked deposition of calcium were not stained blue with the hematoxylin stain, but were pink, indicating thereby that the calcium present in these necroses is either too small in amount to be visible in the stained section or that it is present in a form which cannot combine with the hematoxylin. If the last conception should be correct the stainability of intracellular calcium would depend upon the physicochemical state in which this substance is held. A certain similarity would exist in this respect to the stainability of intracellular fat. As cellular degeneration is often accompanied by an accumulation of fat, correlations may exist between the increase of mineral ash, especially calcium, and that of fat in the degenerating cell (Schultz-Brauns). It is, however, doubtful, if an accumulation of calcium in the cell designates always a degenerative process or if it may also characterize a cell with low vital activities.

PART II. THE EFFECT OF PARATHORMONE AND VIOSTEROL MEDICATION UPON THE MINERAL CONTENT OF NORMAL AND MALIGNANT TISSUES IN RATS

Various authors have attempted to influence cancer growth by the introduction of substances which affect the calcium metabolism, such as calcium salts, Irradiated ergosterol and parathyroid hormone, into the cancerous organism

(Goldfeder,[14] Paik,[15] Barelli,[16] Sugiura and Benedict,[17] Goerner,[18] Goerner and Shafiroff,[20] Sumi,[19] Bischoff and Maxwell,[21] Reding and Slosse[22]). Shear[13] came, in his recent review on this subject, to the conclusion that parathormone treatment is ineffective as an agent which may inhibit or stimulate malignant growth and that vitamin D is without any reliable antigrowth action. This conception is confirmed by observations made by me some time ago on a larger series of cancer patients who received calcium salts, viosterol and parathormone over a prolonged period and in relatively large doses.

In view of the controversial observations made on the blastic action of vitamin D and parathormone, a histochemical study of ashed sections prepared from cancer tissue of animals, treated with these two agents, seemed to be of interest, as it could be assumed that any action of these substances on the tumor would very likely be associated with definite changes in the mineral constitution of the cells.

Six rats, three inoculated with the transplantable sarcoma, Philadelphia No. 1, and three inoculated with the transplantable carcinoma, Walker No. 256, were used in the parathormone experiment. After the tumors had reached the approximate size of a walnut and after a small piece of the tumors had been removed for comparative study, the rats received subcutaneous injections of parathormone. One animal was killed twenty-four hours after the injection of 50 units of parathormone; three rats were killed sixteen hours after the injection of 100 units of parathormone. Rat 5 was sacrificed sixteen hours after injection of 150 units and Rat 6 received a total of 250 units in the course of three days and died on the fifth day after the beginning of the medication.

As the rat is rather resistant to parathormone (Collip,[23] Tweedy and Chandler,[24] McJunkin, Tweedy and Breuhaus[25]), relatively large doses of parathormone are required for the production of toxic degenerative organic lesions, such as those rather readily obtained in dogs (Hueper).

In the viosterol experiment six rats with sarcoma, Philadelphia No. 1, were fed with 0.75 c.c. of viosterol 10,000 X daily by mouth with a dropper. After five days of this medication the rats became listless and showed a coarse fur. The treatment was discontinued and one animal was killed four days later. After an interval of a week the medication was resumed for a period of four days. One animal died two days after the resumption of the feeding and one was killed at that time. The remaining three animals were killed ten days later, while they were still showing symptoms of viosterol intoxication.

Any inhibitory effect of the viosterol medication upon the growth rate of the tumors was not observed. This may be noteworthy, in spite of the small number of animals used, on account of the rapid growth of these neoplasms.

The autoptic examination of the rats did not show any macroscopic pathology in the tumors and organs which might have been referred to the medication given, with exception of Rats 5 and 6, where irregular whitish, soft areas and streaks were found on the cut surface of the kidney and heart, indicating the presence of necroses.

The histologic findings in the organs of the rats in both groups were essentially identical. The following organs were examined: tumor, salivary gland, heart, lung, liver, pancreas, spleen, kidney, lymph node, suprarenal gland, and skeleton muscle.

The stained and incinerated sections prepared from the tumors of the

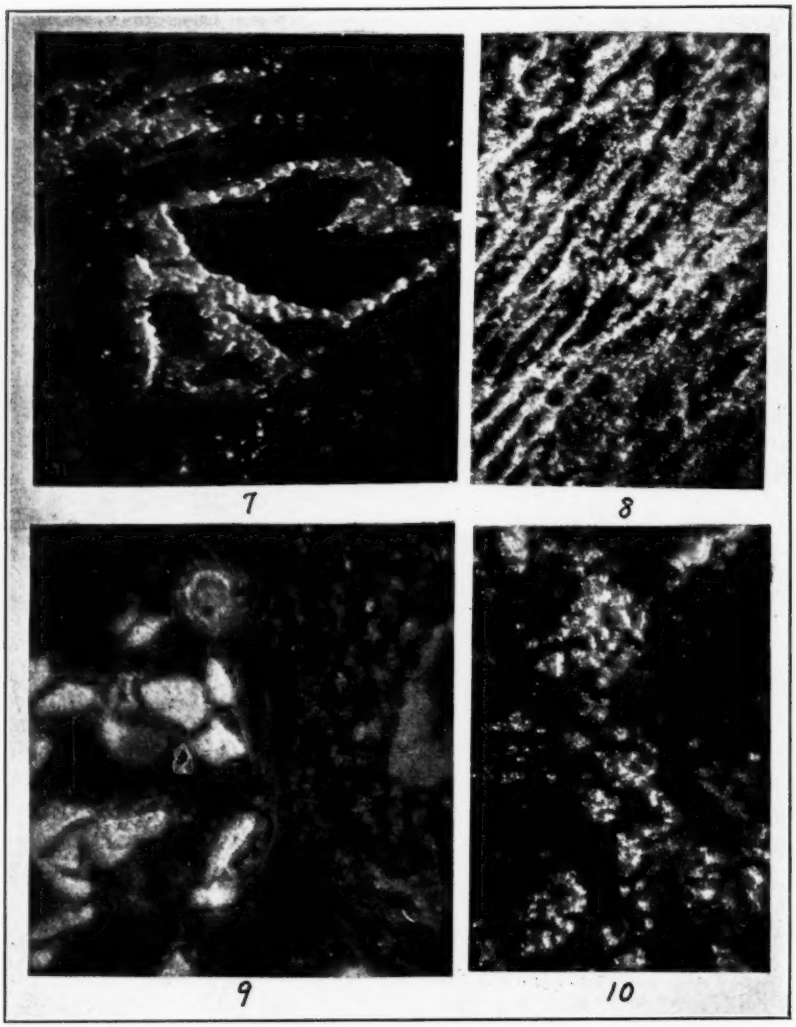

Fig. 7.—Vascular and myocardial calcification in the heart after parathormone poisoning.

Fig. 8.—Accumulation of calcium particles in the myocardium without evidence of degenerative changes in the stained section.

Fig. 9.—Accumulation of calcium in the muscularis of the esophagus of Rat 6, which died from parathormone poisoning.

Fig. 10.—Calcifications in the kidney of Rat 6.

treated animals did not reveal any appreciable and fundamental difference from those made before the medication was started. There were no changes in the stainability of the cytoplasm and of the nucleus of the tumor cells. The ashed sections were essentially identical with those previously described.

There was neither an increase nor a decrease of mineral matter, especially of calcium, in the tumor cells and the intercellular spaces.

In contrast to these negative results in the tumors definite histologic and histochemical pathology was found in some of the organs of the treated rats, especially in Rats 5 and 6, indicating that the medication given had been intense enough to cause pathologic lesions in some parts of the organism.

Heart.—There is a moderate to marked interstitial and especially perivascular leucocytic infiltration of the myocardium. A bluish stained hyaline material is found here and there in the perivascular spaces in between the leucocytic exudate. The walls of the myocardial vessels of Rats 5 and 6 are markedly thickened and deeply blue stained. Reddish and blue stained necroses surround and extend from these vessels into the myocardium. The incinerated sections show a moderate, spotty increase of white reflecting particles. The leucocytic infiltrations appear as small accumulations of strongly reflecting white dots. The vascular walls in the heart of Rats 5 and 6 appear as thick, solid white rings, from which less massive accumulations of white matter extends into the surrounding ash which is also rich in white reflecting granules.

Esophagus.—The muscularis of the esophagus of Rat 6 is dark grayish blue in color and contains dense accumulations of calcium particles in the ashed section.

Liver.—In some of the livers examined the cytoplasm of the Kupffer cells and of scattered individual liver cells is blue stained. The distribution of accumulations of white granules corresponds approximately with that of these bluish stained cells. There is a general increase of white ash. It is noted that the liver cell areas surrounding the central veins do not participate in this increase, but are composed of bluish and reddish reflecting ash.

Kidney.—The tubular epithelium of the cortex shows a distinctly bluish stained substance located in the pink stained cytoplasm. This bluish material surrounds sometimes the nucleus and exudes and fills the lumina of the tubules. Small, isolated foci of necroses are occasionally seen. In the kidneys of Rats 5 and 6 extensive necroses of the tubular epithelium are present. There are also localized calcifications of the necroses and calcium casts in the tubules in great number. The walls of many of the smaller vessels are thickened and intensely blue stained, indicating a high degree of calcification.

The ashed sections show a marked increase of white reflecting material, which often forms dense clumps or rings corresponding in their distribution with that of the tubular necroses and vascular calcifications, respectively, seen in the stained sections.

The microscopic examination of the stained and ashed sections of the other organs mentioned above did not reveal any differences from the conditions seen in the sections of a series of three normal rats of the same strain and approximate age as the experimental animals.

COMMENT

The observations made seem to indicate that the cells of the two rat tumors used do not belong to that group of tissues in which disturbances of the mineral metabolism caused by overdosage of parathormone and viosterol, respectively, result in local degenerations and necroses. The absence of any appreciable changes in the amount, character, and distribution of the mineral matter of the cancer cells following the medication of parathormone and viosterol suggests that the malignant tissue is apparently unable to withdraw calcium from the hypercalcemic blood, because the calcium is present in the blood in a form which cannot be taken up by the malignant cells. This explanation is supported by the observations of Kluge[27] who found, that there is a marked difference in the absorption of calcium compounds by tumor tissue

depending on their organic or inorganic character. While the subcutaneous injection of inorganic calcium compounds resulted in a marked temporary increase of the calcium content of the tumor tissue, its calcium content was barely increased when organic compounds were used.

The presence of a bluish stained substance in the perivascular spaces of the myocardial vessels and in the cytoplasm of the renal epithelium and liver cells together with the corresponding increase of calcium particles in these cells according to the observations made in the ashed sections suggests, that intracellular disturbances of the mineral metabolism precede and cause the degeneration and necroses of the myocardium and kidney occurring in parathormone and viosterol poisoning. This conception receives additional support by previous investigations of Hueper,[7] McJunkin, Tweedy and Breuhaus[25] and Ham.[26] It is obvious that the lethal cellular calcinosis may not always be followed and intensified by an additional necrotic calcification. The presence of a hypercalcemia will, however, favor this process. The contrast existing between the marked increase of calcium in degenerative and necrotic foci, according to the ashed sections, and the absence of a positive staining reaction for calcium in these areas, according to the stained sections, seems to suggest the possibility that the demonstration of calcium in the tissue with the hematoxylin stain depends not only on the amount but also on the character of the calcium compound present.

CONCLUSIONS

1. Microincineration of two transplantable rat cancers showed that the healthy tumor cells consisted almost exclusively of bluish reflecting granules, representing presumably sodium and potassium.

2. Iron and calcium did not seem to play any important rôle in the mineral constitution of these cells.

3. Cellular degeneration in these two tumors was characterized by an increase of the calcium content of the cells, manifested mainly in the formation of white nuclear rings.

4. The character and composition of the mineral skeleton of the tumor cells could not be changed by the medication of parathormone and viosterol.

5. Degenerations and necroses in the kidney and heart of rats receiving excessive amounts of parathormone and viosterol were apparently caused by the toxic action of an intracellular calcium accumulation.

I am indebted for technical assistance to Miss M. Russell, Cancer Research Laboratories, University of Pennsylvania.

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THE SIGNIFIANCE OF STREPTOCOCCI THAT RESEMBLE DIPHTHEROIDS RECOVERED FROM BLOOD CULTURES IN SUBACUTE BACTERIAL ENDOCARDITIS*

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AN ASTONISHING change in size and shape of streptococci may be noticed under different conditions of cultivation.[1] At times these changes result in forms that have a distinct resemblance to the diphtheroid group of bacilli. In old cultures the changes that take place are probably best interpreted as "involution forms." The term "diphtheroid" is applied to an ill-defined group of bacilli, largely on account of their morphologic resemblance to the diphtheria bacillus. In this article the terms "diphtheroid" or "diphtheroid appearing" are used because of the morphologic resemblance of the organisms studied to the diphtheria bacillus and do not specify any particular bacterium.

Jensen and Morton[2] have, from a case of acute cystitis, isolated a streptococcus that under certain conditions resembled a diphtheroid bacillus. They speak of the organism as having a diphtheroid and a streptococcus phase.

Tow and Wechsler[3] have reported a case of acute endocarditis in a girl eleven years old in which diphtheroids were recovered from the blood during life and at autopsy. One year prior to the onset of the acute illness the girl had pain in the right knee and fever which lasted one week. Eight blood cultures during the course of the disease were negative, and the ninth was positive for a diphtheroid organism. Sections of the vegetations taken at autopsy showed cocci in pairs and groups. The vegetations were not cultured. In their conclusion Tow and Wechsler state that the existence of diphtheroid endocarditis is problematical and that it is likely that the streptococcus was the etiologic agent and the diphtheroid a secondary invader.

According to Thomson and Thomson[4] "true diphtheroids" do not show streptococcal forms in broth cultures. If this statement is true then it is of unusual interest to find diphtheria-like forms and typical streptococci in smears from uncontaminated young broth cultures from the blood of a patient suspected of having subacute bacterial endocarditis; further to find similar forms in a (Gram-Weigert) stained section from the vegetations on the heart valve when the patient came to autopsy.

REPORT OF CASES

CASE 1.—*History:* L. H., twenty-three years of age, admitted to The Meriden Hospital, Jan. 31, 1933, complaining of weakness and fever. Had rheumatic fever in 1921. About two weeks prior to admission, patient had grip, on fifth day pain developed in right shoulder,

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and persisted for three or four days, then disappeared. Had one attack of nosebleeding before admission. Had severe headache. Had palpitation and dyspnea occasionally. *Examination:* Throat slightly injected. Tongue coated. X-rays showed one abscessed tooth. Heart enlarged to the left; the point of maximum impulse was in the sixth interspace anterior axillary line. A presystolic thrill was felt. Diastolic murmur along the left border of the sternum most marked at the apex and referred to the left axilla. Lungs were clear. Spleen was felt at the costal margin. On February 14, embolic spots were found on finger tips. February 18, abscessed tooth was extracted. From this was cultured nonhemolytic, green pigment-producing streptococcus.

In view of favorable reports by Brody and Crocker,[5] Gordon,[6] Stephenson[7] and Howell, Portis and Beverly,[8] an immunotransfusion was decided upon. A vaccine was prepared from the organism found in the patient's blood stream. A suitable donor was given six injections of the vaccine subcutaneously, and one injection intravenously. Following the last injection he had a severe reaction with chills and fever.

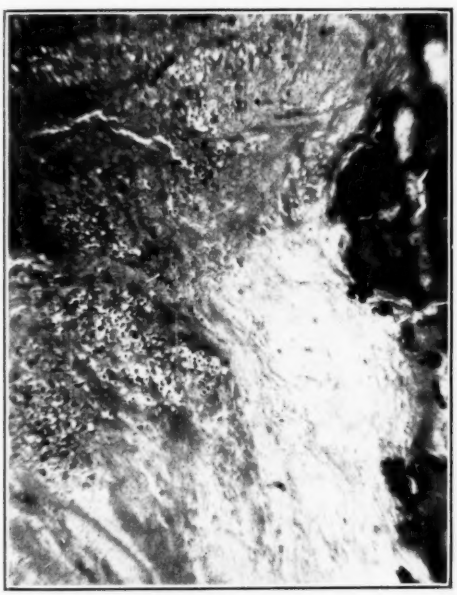

Fig. 1.

Fig. 2.

Fig. 1.—Shows enormous masses of bacteria in the vegetation on the aortic valve. Gram-Weigert stain. ×100.

Fig. 2.—Section from the vegetation on the aortic valve stained by Gram-Weigert method. Shows an abundance of diphtheroid organisms and one chain of streptococcus. ×1000.

Six days after the last injection was given to the donor, the patient was given 300 c.c. of whole blood. Following transfusion he developed cyanosis, dyspnea, and cough. This critical condition persisted for several hours. He was slightly dyspneic for two or three days. From then on his condition became gradually worse, and he died on March 20. The temperature was irregularly high, ranging from 90° to 104° from admission until four days before death, when it fluctuated between 96° and 98°.

Laboratory Observations: Wassermann negative. Blood nonprotein nitrogen 24 mg. Blood sugar 120 mg. Several blood counts were done. The erythrocyte count varied between 4.4 millions on February 1 to 3.6 millions on March 6. The leucocytes ranged from 12,200 on February 1 to 16,600 on March 6. The differential remained fairly constant. Hemoglobin 75 per cent; polymorphonuclear neutrophiles 74 per cent; small lymphocytes 17 per cent; large lymphocytes 12 per cent. The blood culture was positive.

AUTOPSY

Gross Observations: Well-developed and nourished adult male. Petechiae observed on extremities and abdomen. Finger nails, lobes of ears and lips deep bluish in color. Each pleural cavity contained about 500 c.c. of clear, straw-colored fluid and a few flakes of fibrin.

Heart: Weighed 500 gm. The myocardium was soft and pale. The pulmonary and tricuspid valves appeared normal. The free margin of the mitral valve was slightly thickened and whitish in color. There were three pale grey, friable vegetations measuring 0.3 cm. in height by 0.3 cm. in diameter at the base of the right cusp of the mitral valve. The vegetations were located on the ventricular surface of the valve. The columnae carnae were flattened and some chordae tendineae showed slight fibrosis. The cusps of the aortic valve were obscured by a large, pale grey, friable vegetation. All the cusps were involved. The great mass of the vegetation was on the ventricular surface of the valve. The cusps were not ulcerated, although they were considerably distorted in shape. The remaining organs showed the usual appearance as found in subacute bacterial endocarditis.

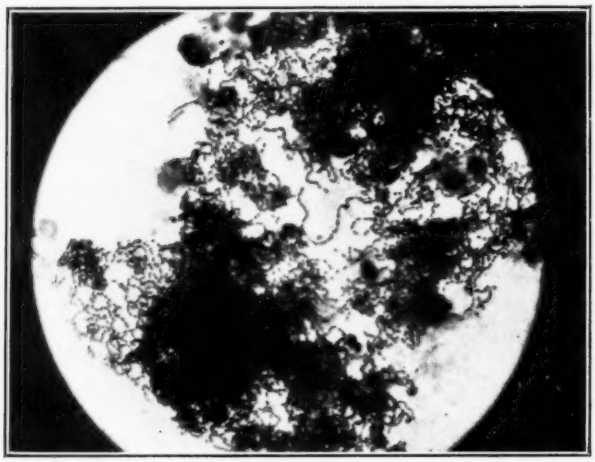

Fig. 3.—Smear from broth culture which was planted from a blood agar plate. Shows a marked predominance of long chain streptococcus. ×1000.

MICROSCOPIC EXAMINATION

Section through the aortic valve showed a massive vegetation composed of polymorphonuclear leucocytes, fibrin, platelets, red blood cells, and dark blue staining masses of bacteria. A Gram-Weigert stained section from aortic valve showed great masses of cocci in chains and diphtheroid appearing organisms. The microscopic examination of the remaining organs showed nothing of special importance.

Summary: The important findings in this autopsy were vegetative endocarditis involving both aortic and mitral valves, multiple petechiae over the body, infected infarcts of the spleen, chronic passive congestion of the liver and acute congestion of the lungs, spleen, and kidneys.

CASE 2.—*History:* F. G., unmarried female, nineteen years of age, was a dramatic art teacher. The significant features in the past history were scarlet fever at seven and chorea at ten years of age. Her last illness began in September, 1932, when she complained of pain in hips and ankles and went to bed for three weeks at home. On getting up she had pain in her back and had an enlarged liver and spleen.

She was a patient in the New Haven Hospital, New Haven, Conn., in November, 1932. A blood culture on Nov. 3, 1932, was positive for diphtheroids. The patient left the New Haven Hospital in December, 1932, against advice. The diagnosis on the record was

subacute bacterial endocarditis.* She was admitted to the Meriden Hospital, Dec. 29, 1932, and discharged Jan. 14, 1933, on request of the family. During this period four blood cultures were taken with negative results. Following discharge from the hospital, patient was admitted several times for blood transfusions and usually remained in the hospital a day each time. On April 25, 1933, a blood culture was positive for diphtheroids. The bacteriology of these diphtheroid-like organisms was similar to the bacteriology of those described in the case reported above. Patient died in May. An autopsy was refused.

BACTERIOLOGIC STUDIES

Three cultures were studied in this series. The first (laboratory number 3,674) was isolated from a blood culture during life in patient in Case 1, the second (laboratory number 7,038) was isolated from heart's blood at autopsy; the third (laboratory number 14,722) was isolated from blood of patient in Case 2 during life. After isolation several platings on blood agar were made and in each instance single colonies chosen to rule out possibility of contamination. Culture (laboratory number 14,722) was the same in all respects as Culture 3,674, so for the sake of brevity a description of the experimental studies on this culture are omitted.

Morphology.—The morphology of all three cultures was studied when grown on Loeffler's medium, blood agar plates (both aerobic and anaerobic) and in Douglas broth.

On Loeffler's medium culture 3,674 was a mixture of gram-positive streptococci in association with diphtheroid, bacillary-like forms (narrow, club-ended, and granular). There was a tendency for the latter forms to decolorize with the Gram stain. Culture 7,038 was similar microscopically except that the streptococci and paired forms predominated in young cultures. Cultures five days old, however, showed many diphtheroid forms.

The growth on blood agar plates of Culture 3,674 (aerobic and anaerobic) was fine and veil-like in character. In forty-eight hours a small zone of hemolysis appeared. By repeated incubation at 37° C. followed by refrigeration, secondary hemolytic rings were developed. The hemolysis was typical of the alpha variety.[9] Both diphtheroid and streptococcus forms were demonstrated microscopically.

The beta type of hemolysis was produced with Culture 7,038 when grown on blood agar plates either aerobically or anaerobically. Individual colonies were larger than with Culture 3,674 and showed wide, clear zones of hemolysis. After a series of eight daily transplants a few alpha type colonies developed which retained their alpha characteristics on subculture. Both diphtheroid and streptococcus forms were demonstrated microscopically.

Transplants to Douglas broth caused practically complete change to the streptococcus phase of both Cultures 3,674 and 7,038 regardless of whether inoculum was obtained from Loeffler's slants or blood agar plates. Continued daily transplants over a period of forty days were made and without exception a pure culture of long-chained streptococci (16-18 cocci to a chain) was obtained. The streptococci remained constant in their morphology for three

*We are indebted to the Department of Internal Medicine of Yale University for permission to use data from records of the New Haven Hospital.
The microphotographs were taken by Mr. Jean Kiefer of The Norwich Tuberculosis Sanatorium.

to four days. Usually between the fifth and seventh day diphtheroid forms could be demonstrated microscopically as the chains of streptococci appeared to have changed to the diphtheroid phase. Transplants at this point to fresh Douglas broth, however, again resulted in a pure culture of streptococci. There was a general trend toward the diphtheroid phase in cultures as they grew older. In no instance was the reverse of this phenomenon noted in any medium upon aging.

Biochemical Reactions of 3,674 *and* 7,038.—Both cultures were inoculated into 1 per cent sugar broths from Douglas broth, Loeffler's slants, and blood agar slants. Sugar broths were sterilized by filtration. Both cultures, 3,674 (alpha hemolytic) and 7,038 (beta hemolytic), gave similar reactions: Dextrose, lactose, sucrose, salicin and maltose were fermented with the production of acid. Mannite, rhamnose, arabinose and raffinose were not affected although definite growth was obtained. In litmus milk, acid and coagulation was produced. On the basis of the morphologic and biochemical reactions Culture 3,674 would be classified as a strain of *Streptococcus viridans*, variety probably *mitis*. Culture 7,038 would be classified as a strain of *Streptococcus hemolyticus*.

SEROLOGIC STUDIES

In order to augment our findings both cultures were sent on blood agar slants to the Rockefeller Institute* for further study. The following report was obtained:

"Culture 7,038 seems to be a typical hemolytic streptococcus. A precipitin test with an extract of the organism shows that it contains the carbohydrate 'C,' characteristic of strains of human origin (Group A).

"Culture 3,674 seems to be a typical *Streptococcus viridans* and not merely a non-hemolytic form of 7,038, since extracts of 3,674 do not show any traces of the 'C' substance characteristic of hemolytic streptococci belonging to Group A."

After several weekly transplants on Loeffler's slants and blood agar plates, Culture 3,674 developed colonies which showed typical beta hemolysis. When single colonies were chosen and plated on blood agar, however, both alpha and beta type colonies developed. Morphologically and biochemically both types of colonies (alpha and beta) agreed with the colonies obtained from Culture 7,038 (alpha and beta). These four cultures were then sent to the Rockefeller Institute and tested for the group carbohydrate "C." The two beta hemolytic strains both gave precipitin reactions with anti "C" serum for Group A and no reaction with anti "C" serum for any of the other groups. The two alpha hemolytic strains did not react with any of these sera. From these studies it is obvious that the colonies showing alpha hemolysis are antigenically distinct from those showing the beta type.

COMMENT

The interesting features of the case clinically were: the reaction following transfusion with a specifically immunized donor; and the blood culture studies.

*The authors are indebted to Dr. R. C. Lancefield for these studies.

Two possibilities present themselves as probable causes of the severe reaction following immunotransfusion. First, the occurrence of a pulmonary embolus following the transfusion, always a possibility, must be considered. Second, the interaction between the antibody of the donor and antigen of the recipient. Of these two possibilities, the latter appears to us to be the more likely. The patient's tissues were proliferated with antigen. Both the streptococcus and diphtheroid phases were demonstrated in tissue section at autopsy. The donor received both phases in the vaccine used for production of the therapeutic serum. With a preponderance of antigen in his tissues, the recipient was in a highly sensitive condition and the violent reaction following transfusion with whole blood containing antibody was probably due to an antigen antibody complex in the tissues and blood stream of the patient. The clinical picture of cyanosis, dyspnea and cough directly following the immunotransfusion would appear to augment this theory.

Jensen and Morton's[2] experiments with the diphtheroid phase of a streptococcus isolated from a case of acute cystitis led them to believe that the diphtheroid phase was nonpathogenic. We are carrying on experiments at the present time that appear to show that in rabbits the diphtheroid phase is pathogenic in the sense that definite valve localization can be produced. At this time, however, we wish to suggest the possibility that in certain instances, in subacute bacterial endocarditis, it is the attenuated diphtheroid phase of the streptococcus that first becomes implanted on the heart valve. Once a small clot is formed on the valve the medium is perhaps suitable for the change of the organism into the streptococcus phase following which the disease progresses in the usual manner.

In a study of 1,079 blood cultures Thompson[10, 11] has shown the presence of diphtheroids in 3 per cent of those examined. His object was to test the hypothesis advanced by Koch and Mellon[12] that diphtheroids are found in the blood stream in persons convalescent from streptococcal infections. Thompson believes that diphtheroids in blood cultures appear to have the same significance as the saprophytic cocci.

Thompson[10, 11] states that "Any organism which appears as rods, and which in subcultures in broth develop chains of streptococci may better be classified as streptococci, especially if it is not possible subsequently to bring about the reverse change from the streptococcal to the rodlike form." The organism numbered 3,674 in this investigation originally showed a majority of diphtheroid forms and subsequently developed into a streptococcus, but we have been able to change this organism back to the diphtheroid phase at will by the use of proper media.

If we accept the hypothesis of Thomson and Thomson[4] the organism would have to be classified as a streptococcus with a diphtheroid phase. However, whether we regard this phenomenon as one of pleomorphism or not is not our basic interest. We are primarily interested in focusing attention on the significance of the finding of diphtheroid appearing organisms in blood cultures, particularly in cases of subacute bacterial endocarditis.

Grinnell[13] reports that he was able to obtain from single cell strains of *Streptococcus hemolyticus*, strains of *Streptococcus viridans* with constant char-

acteristics. He notes "the alpha variants remain true to type, the loss of hemolytic power is accompanied by a slight loss of virulence, the alpha derivatives usually but not always give the same fermentative reactions." In this investigation we were able to confirm these results. The alpha variants from the beta hemolytic types were antigenically distinct although giving identical fermentative reactions.

Our experiments on animals to date have shown definitely that the organism has very low virulence. Large quantities, regardless of the phase, when injected into rabbits produce no toxic effect. However, by repeated injections of any of the three phases over a period of from twenty-three to thirty-five days definite localization has been demonstrated. This section of the investigation has not yet been completed.

SUMMARY

1. Two cases of subacute bacterial endocarditis are described in which diphtheroid appearing organisms were recovered repeatedly from blood cultures during life.

In Case one, Gram-Weigert stained sections from the vegetation on the aortic valve at autopsy showed both diphtheroid appearing forms and chains of streptococci in each section. Bacteriologic investigation in both cases showed that the streptococcus under study existed in two phases, a typical streptococcus phase and a diphtheroid phase.

2. As a result of the bacteriologic studies of the two cases reported in this paper evidence is submitted which shows that the occurrence of diphtheroid appearing organisms in blood cultures from suspected cases of subacute bacterial endocarditis must be regarded as important. The organism, isolated from blood cultures in the diphtheroid phase, changed under suitable conditions into a green producing streptococcus which is unquestionably of etiologic significance.

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THE USE OF GALACTOSE IN THE DIFFERENTIAL DIAGNOSIS OF JAUNDICE*

A Preliminary Report

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IN A SURVEY of the literature concerned with the physiology of the liver and the tests devised to estimate its functional capacity,[1, 2, 3, 4, 5, 6] it is noteworthy that until recently very little of clinical value has been accomplished either in the diagnosis or in the prognosis of liver disease.

There are several reasons for this. First, the liver is a huge organ, the site of multiple activities, and has been aptly called "the great storage battery of the human body." It is obvious, therefore, that no one test is likely to yield an accurate index of its total functioning capacity. Furthermore, experimental work has shown that there is a tremendous hepatic reserve, a reserve greater probably than in any other organ of the body. To quote from the report of Bollman and Mann on lesions of the liver produced experimentally in dogs:[5] "It would appear that any test, capable of measuring the metabolic changes which depend entirely on the liver, would give an accurate index of the efficiency of this organ. There are several reasons why such tests show but little, and studies of animals from which the liver has been partially removed reveal some of the reasons for the failure of those tests. In the dog and in many other animals, the liver consists of several distinct lobes, one or more of which may be successfully removed. Immediately following such operations, the remaining portions of the liver begin to hypertrophy, and by serial operations, removing a lobe of liver every few weeks, it is possible to remove more hepatic tissue than was originally present and still have a normal amount of hepatic tissue remaining in the dog. During this process no signs of hepatic insufficiency are observed except a transient upset of a few days' duration, which is probably due to injury of its remaining hepatic tissue rather than to actual deficiency because of removed tissue. . . . It is obvious that less than 20 per cent of liver can accomplish the work of the entire organ."

Second, the main effort and emphasis in the study of this problem from the laboratory standpoint have been expended on attempting to estimate the excretory capacity or permeability of the liver by the use of various dyes—in a way analogous to the dye tests of renal function. As a result of this, the bromsulphalein and rose bengal tests have come into general clinical use. Their value is indeed very limited, for while the rate of their elimination from the blood stream and excretion by the liver may give some information as to the permeability of the liver and the patency of its biliary system, it helps not at

*From the Cleveland Clinic.
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all in estimating the ability of the liver to perform its more vital functions. Foremost among these may be mentioned the conversion of sugar to glycogen, its storage and release again into the blood stream as glucose; the synthesis of urea; the formation of bile pigment and the detoxification of poisons.[6] The dye excretion tests do not indicate the capacity of the liver to perform these functions; and furthermore, when either intrahepatic or obstructive jaundice is present, the estimation of dye in the plasma is bound to be inaccurate, yielding a value which is almost always too high.

Tests of pigmentary function of the liver, of which the van den Bergh test is outstanding, have been of no help in determining the nature or extent of liver damage and have failed particularly in the differentiation of obstructive and intrahepatic jaundice. In recent years, the rate of removal of bilirubin injected intravenously has been used as a test of liver function as reported by Bergmann in 1927 and by Harrop and Barron in 1931.[7]

Estimation of the cholesterol content of the blood plasms in cases of hepatic and biliary disease has been reported recently in an excellent article by Epstein.[8] This promises to be a valuable means of estimating liver damage.

Since the discovery of the glycogenic function of the liver by Bernard about the middle of the last century, many reports have been published in the literature on the utilization of various sugars for testing hepatic function. These investigations have been productive of widely divergent results and interpretations, due to the fact that the tests were not standardized, that they were used in all varieties of liver disease, and that no allowance was made for the properties of great reserve and rapid regeneration which we now know to exist. In short, extensive acute or subacute damage to the liver must be present before changes are reflected by a derangement of carbohydrate metabolism.

In this report, I shall confine the discussion to that puzzling group of cases of relatively painless acute jaundice in which neither the history, physical signs, nor laboratory reports make the diagnosis clear. These cases may be due to common duct obstruction of one sort or another or may be caused by acute or subacute diffuse liver damage, such as occurs in catarrhal icterus or toxic hepatitis.

Recently a modification of the glucose tolerance test has been used with some success to estimate the liver function by workers at the University of California.[9]

Keeping in mind the functional complexity of the liver, its great reserve and power to regenerate rapidly, and the present inadequacy of the tests in general use to determine its "vital capacity," the problem of jaundice and its differential diagnosis by means of galactose tolerance may be considered.

This differentiation has often confused the keenest clinicians, yet it is of the utmost practical importance to the patient, since the therapeutic measures are diametrically opposed. Extrahepatic obstructive jaundice is a surgical problem. In jaundice due primarily to inflammation or degeneration of the liver parenchyma, surgery is positively contraindicated. The galactose tolerance test of liver function furnishes a simple and positive means of making this differentiation.

USE OF GALACTOSE

About thirty years ago the use of levulose as a test for liver function was attempted by Strauss, and five years later Richard Bauer of Vienna began to use galactose for the same purpose.[10] Since that time levulose, dextrose, and fructose all have been proposed and tried as tests of the carbohydrate metabolism of the liver with little success. Galactose, which appears to be the most valuable of the monosaccharides for this purpose, has received but little attention in this country.

In 1931 several papers were published by Shay, Schloss, Bell, and Rodis of Philadelphia outlining the theoretical considerations for the use of galactose as a test of liver function[11] and also their results in cases of jaundice.[12] The work here reported was done in order to confirm or deny their findings.

From a theoretical standpoint, in considering the use of any carbohydrate as a test for liver function, galactose seems best suited for this purpose. First, the sugar must be pure and stable. When given orally it must be absorbed from the gastrointestinal tract easily and with little decomposition by bacteria. It must be capable of transformation by the liver into glycogen, preferably with difficulty; the normal tolerance for the sugar must be known; and finally, there must be a stable renal threshold.

Galactose may be obtained in a pure form, though it is rather expensive. It is rapidly absorbed from the gastrointestinal tract, and, according to present knowledge, it is utilized *only* by the liver and that with difficulty. It is well known that the other sugars have no such specificity. Furthermore, experimental work has shown that after reaching the general circulation it is all excreted by the kidneys, regardless of the condition of the other glands. There is no renal threshold for galactose.[11]

Since the liver has such adequate reserve and such power for rapid regeneration, it is clear that in local or slowly progressive liver disease there is no change in the carbohydrate metabolism. But it is equally obvious that marked derangement of carbohydrate metabolism might be expected when there is extensive acute or subacute damage to the liver cells or when chronic damage has gone on to such a stage that regeneration fails. The variable results which have attended many types of tests for the function of sugar metabolism may be explained partly on this basis. In other words, the test has definite limitations, and it is these very limitations which make it valuable in the differential diagnosis of jaundice. The galactose tolerance test of liver function serves to identify and differentiate those cases of diffuse liver damage which occur in toxic or infectious jaundice from those cases of jaundice due to obstruction of the bile ducts.

TECHNIC OF THE GALACTOSE TOLERANCE TEST

The work of Shay and Schloss has shown that the normal liver, regardless of age, sex or condition of the other glands, can metabolize about 40 grams of galactose. That portion not stored by the liver appears in the urine as a reducing substance, identified chemically and polariscopically as galactose. In their series of normal persons, this output varied from 0 to 3 grams.

The test is carried out as follows:

1. A fasting specimen of urine is obtained.

2. The patient is given 40 grams of pure galactose, dissolved in one pint of water.

3. Breakfast is omitted.

4. All urine voided in the next five hours is collected, preferably in hourly specimens.

5. The specimens are tested qualitatively for galactose and all those positive are lumped in one volume and tested quantitatively by the Benedict method. (In diabetic patients it is possible to eliminate glucose by the process of fermentation with yeast, as galactose is relatively stable.)

In Table I are listed the series of normal values prepared by Shay and Schloss and my own series of normal values, obtained by the same technic. In respective columns are given the ages of these persons in years, their weight

TABLE I

COMPARATIVE STUDY OF EXCRETION OF GALACTOSE IN MALES AND FEMALES

Shay et al.

FEMALES			MALES		
AGE IN YEARS	WEIGHT (POUNDS)	GALACTOSE IN URINE (GM.)	AGE IN YEARS	WEIGHT (POUNDS)	GALACTOSE IN URINE (GM.)
5	52	0.50	6	36	0.50
26	100	0.00	52	110	1.20
55	102	0.25	64	110	1.50
64	107	2.20	53	112	1.08
63	108	0.69	59	115	0.30
55	110	0.13	42	120	2.20
42	110	1.00	30	126	1.00
63	115	0.30	64	129	0.00
49	115	0.70	50	129	1.20
64	134	0.98	50	133	1.10
35	150	0.00	40	142	1.20
18	153	0.65	69	160	0.00
38	162	1.98	59	162	1.10
50	175	0.00	51	162	1.87
32	185	0.19	54	166	0.00
Average		0.64	Average		0.88

Author

20		0.0 (trace)	1.10
↓		0.68	1.50
		0.96	1.20
		1.30	0.70
		2.00	0.82
		1.47	0.68
		1.47	1.50
		0.00 (trace)	1.65
		0.00	0.00
		0.75	0.70
		0.40	0.50
		2.10	1.50
		0.85	0.50
↓		0.75	0.80
70		0.00	0.40
Average		0.85	0.90

in pounds, and the amount of galactose found in the urine during the five hours following ingestion.

It is seen in these two series containing sixty normal cases that the average urinary output of galactose is less than 1 gm. The highest output in the first series was 2.2 gm. and in my series, 2 gm.; while the great majority of normal persons excreted less than 1 gm.

From these observations it seems safe to state that an output of 3.0 gm. or more is abnormal. In my series it has been found invariably that in normal persons galactose, if excreted at all, appears in the first and second specimens and rarely is found in the later specimens.

At the Cleveland Clinic, galactose has been used as a test of liver function for over a year. The results of this investigation may be summarized as follows: From the standpoint of the amount of galactosuria, the patients tested fall into two general but rather definite groups. In those excreting *abnormal* amounts of galactose, the clinical impression pointed toward diffuse liver damage; in those with a *normal* output, clinical investigation or subsequent exploration revealed the jaundice to be due to biliary obstruction. A summary and comparison of clinical and operative findings with the results of the galactose tolerance test are presented. (Table IIA and IIB.)

In the first group of undoubted cases of biliary obstruction, the galactose test, prior to operation, showed in each of these a normal secretion, averaging 0.87 gm.

In the second group, however, in which the diagnosis of diffuse liver damage is reasonably certain, marked galactosuria ranging from 1.90 to 9.1 gm. (average over 5 gm.) was present. These results correspond very well with the observations of Shay, Schloss and Rodis.[12]

A review of the histories of several of these patients gives some impression of the value of the test in differential diagnosis.

CASE 1.—A man, aged fifty-four years, was admitted to the hospital, complaining of hunger pains, itching, and jaundice of one week's duration. The past history was irrelevant except that he had noticed a gradually increasing epigastric pain before meals during the preceding year. Physical examination revealed moderate, generalized icterus, fair nutrition, and an enlargement of the liver, which was described as being 6 cm. below the costal margin, rough, irregular, and not tender. The cholecystogram showed a nonfunctioning gallbladder. The bromsulphalein test showed 80 per cent retention of dye in one-half hour. The clinical diagnosis was "common duct obstruction, probably stone." The galactose test showed an output of 1.8 gm., corroborating the impression of obstruction. Operation revealed an inoperable cancer at the head of the pancreas.

CASE 2.—The patient, a man, aged forty-two years, was admitted to the clinic, complaining of indigestion, belching, progressively increasing jaundice, and the loss of 10 pounds in weight in the course of three weeks. The patient had had several phallic sores and a positive blood Wassermann two months before, for which he had had no treatment. The patient was well nourished, moderately jaundiced, with a slow pulse rate and slight tenderness over the right upper quadrant. The liver was palpable 3 cm. below the costal margin. The Wassermann test was weakly positive, the bromsulphalein test showed a retention of 30 per cent in half an hour and the galactose test an output of 3.3 gm.—confirming the clinical impression of catarrhal jaundice or syphilitic hepatitis.

CASE 3.—A housewife, aged twenty-six years, came to the clinic complaining of jaundice, weakness, and itching. Four years before, the gallbladder had been removed. Two years

TABLE II

SUMMARY AND COMPARISON OF CLINICAL FINDINGS WITH THE GALACTOSE TOLERANCE TEST

A. *Jaundice of the Obstructive Type*

AGE	DIAGNOSIS	BASIS FOR DIAGNOSIS	DURATION OF JAUNDICE	BROMSULPHALEIN TEST	VAN DEN BERGH TEST	ICTERUS INDEX	GALACTOSE SECRETION (GM.)
54	Carcinoma—pancreas	operation	6 weeks		12.5	100	1.80
45	Carcinoma—pancreas	operation	?		indirect	50	1.47
21	Stricture—common duct	operation	6 months*		11.0		0.00
58	Stone—common duct	operation	4 months*		20.0	60	0.50
21	Biliary fistula	operation	2 months*		16.0		0.50
50	Carcinoma—hepatic duct	operation	6 weeks		18.0		1.30
26	Stricture—common duct	operation	8 months*			75	0.63
55	Stricture—common duct	operation					0.85
		autopsy					
44	Obstructive jaundice	clinical	5 days		12.8	50	0.83
54	Stricture—common duct	operation	6 months*		2.7		0.96
						Average	0.87

B. *Jaundice Due to Hepatitis or Hepatic Degeneration*

56	Hyperthyroidism	clinical	? weeks	100- 60%		50	3.20
41	Cirrhosis—ascites	clinical	2 weeks(?)	100- 80%	6.8	100	6.00
53	Cirrhosis—ascites	clinical	? weeks	100- 90%			6.00
30	Arsenic hepatitis	clinical	1 week	100-100%		100	9.10
43	Catarrhal jaundice or syphilitic hepatitis	clinical	3 weeks	100- 30%	9.0		3.30
32	Cirrhosis	autopsy	6 weeks	80- 50%	5.8	75	4.70
66	Catarrhal jaundice	operation	3 weeks		5.8		4.10
56	Catarrhal jaundice	clinical	2 weeks	100-100%	19.7		7.00
42	Catarrhal jaundice	clinical	10 days	50- 30%			3.90
70	Cirrhosis(?) malignancy(?)	clinical	3 weeks				4.95
36	Arsenic hepatitis	clinical	6 weeks (faint)				1.90
50	Cinchophen hepatitis	clinical	1 week			100	6.00
27	Catarrhal jaundice	clinical	2 days		15.0	100	5.00
62	Catarrhal jaundice	clinical	1 week			75	8.00
						Average	5.22

*Intermittent.

later the patient had jaundice and was operated on again. At that time a small stone was removed from the common duct with subsequent drainage of bile for ten weeks after the operation. Eight months previous to her visit to the clinic, jaundice again had appeared in intermittent attacks with recurrent pain in the left upper quadrant, which pain had persisted. Examination revealed emaciation, generalized icterus, and a large tender liver, with questionable splenic enlargement. The patient had a slight fever, and a slow pulse rate.

The bromsulphalein test showed 60 per cent retention in one-half hour. The icterus index was 75. The clinical impression was that the jaundice was due either to common duct stone or to adhesions. The galactose test showed a normal excretion of 0.63 gm. At operation the common duct was found occluded by adhesions. The liver was normal.

CASE 4.—A woman, aged thirty-two years, was admitted to the hospital complaining of jaundice and weakness and with the story of having had nausea and vomiting, recurrent dull pain in the right upper quadrant, and progressive jaundice of three weeks' duration. Physical examination revealed a poorly nourished young woman with moderate icterus and tenderness over the right lobe of the liver. The cholecystogram showed a functioning gallbladder. The van den Bergh test was direct; the serum bilirubin was 5.8 gm. The clinical impression was "stone in the common duct." The galactose test showed an ouput of 4.1 gm. —indicating an intrahepatic process, rather than obstruction of the duct. Operation for stone did not disclose any obstruction. The appendix was removed, and the patient recovered uneventfully.

This is an excellent example of the value of the galactose test before operative procedures are considered too hastily.

CASE 5.—A dentist, fifty-six years of age, was admitted to the hospital complaining of weakness and slight indigestion for three months. Three weeks before admission he had begun to experience vague epigastric distress and pain in the right upper quadrant, some nausea and clay-colored stools and, ten days before admission, the development of a steadily deepening jaundice. The physical examination revealed generalized icterus, fair nutrition, normal temperature, and a large, tender liver. The gallbladder was nonfunctioning, according to the roentgenogram. The bromsulphalein test showed a retention of 100 per cent, the serum bilirubin was 20 mg., and the galactose output on two occasions was 7.0 and 6.5 gm., respectively. There was much debate as to the nature of jaundice in this case. Operation was seriously considered, but since the jaundice seemed to be subsiding, this was abandoned. The galactose test revealed that the cause of jaundice was intrahepatic, probably of catarrhal nature, and not obstructive.

In reviewing these five representative cases, it is evident that the ability or failure of the liver to metabolize the galactose has given valuable negative evidence as well as positive findings in cases in which operative interference has been considered.

From the standpoint of prognosis, the test also is of value. Several of the patients with catarrhal jaundice or toxic hepatitis have had subsequent galactose tests and concomitant with clinical improvement, and in several instances even before improvement was obvious clinically, the excretion of galactose gradually fell to normal. These patients all recovered. (Table III.)

It has been of interest to review in the literature the cases studied with this test. In his early work, Bauer thought that he had found a test for cirrhosis of the liver. Two years later he reported the finding of an alimentary galactosuria in catarrhal jaundice and described ten such cases showing galactosuria which gradually decreased as the patients improved.[13] He considered an output of more than 3 gm. as indicating a pathologic condition of the liver.

In the comparative series of cases recently published by Shay, Schloss and

TABLE III

PROGRESS STUDIES OF GALACTOSURIA

DIAGNOSIS	DURATION (DAYS)	GALACTOSE EXCRETION (GRAMS)	OUTCOME
Catarrhal jaundice	2	5.0	
	5	3.3	
	29	0.4	Well
Cinchophen hepatitis	7	6.0	
	11	4.0	
	14	4.2	
	17	3.8	
	21	3.0	Improved
Arsenic hepatitis	7	9.1	
	21	2.3	Well
Catarrhal jaundice	14	7.0	
	15	6.5	
	21	2.0	Well

Rodis, an output of galactose of more than 3 gm. was considered indicative of liver damage, and these authors expressed the thought that the element of chance may work against the test in both of these types of cases. For in mild catarrhal jaundice or hepatitis the carbohydrate metabolism may be only slightly altered and may tend to rapid recovery, while in the other group in which there is chronic damage to liver cells, destruction may progress to a state of diffuse involvement. It is also known that a so-called catarrhal jaundice or hepatitis may complicate the course of a chronic liver disease, such as cirrhosis.

In the series here reported, I have seen and listed several such instances of advanced cirrhosis with ascites and the terminal development of jaundice. In all of these cases, there was an abnormal excretion of galactose. In the average uncomplicated case of cirrhosis, however, assuming that only fibrosis is present, it is obvious that the carbohydrate metabolic function of the liver is normal and that only the permeability or excretory function is deranged. (Table IV.)

TABLE IV

GALACTOSE EXCRETION IN FOCAL OR CHRONIC LIVER DISEASE

AGE	DIAGNOSIS	ASSOCIATED CONDITION	BASIS FOR DIAGNOSIS	BROMSULPHALEIN	GALACTOSE EXCRETION
48	Carcinoma stomach	Metastases—liver	Autopsy	30-20%	1.78
63	Cirrhosis	Diabetes—ascites	Clinical	100-50%	3.82
62	Cirrhosis	Ascites	Autopsy	100-80%	1.27
49	Cirrhosis	Hyperthyroidism	Autopsy	-------	0.00
?	Cirrhosis	----------	Autopsy	-------	trace
48	Carcinoma—site (?)	Metastases—lung, peritoneum	Clinical	-------	0.82
	Nephrosis	Edema	Clinical	-------	0.50
21	Banti's	? Cirrhosis	Clinical and operation	-------	trace
50	Cardiac	? Cirrhosis	Clinical	60-20%	0.84
	Pernicious anemia	Parkinsonism	Clinical	-------	trace
52	Toxic hepatitis	Jaundice (2 months)	Operation Pathologic section	80-80%	trace

To recapitulate—practically all of the cases which have been reported as showing an abnormal excretion of galactose fall into the group in which it is reasonable to believe that a relatively acute diffuse liver damage has taken place. Among these may be listed cases of catarrhal jaundice, arsphenamine hepatitis, phosphorous poisoning, acute yellow atrophy, syphilitic hepatitis, and the hepatitis of infection or intoxication, such as is occasionally seen in pneumonia or hyperthyroidism.

In conclusion, it seems that the galactose tolerance test of liver function furnishes a simple but valuable procedure which clearly identifies and differentiates the toxic or infectious type of jaundice from the jaundice due to extrahepatic causes.

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LABORATORY METHODS

THE DETERMINATION OF SERUM PROTEIN BY MEASUREMENT OF THE VOLUME OF PRECIPITATE*

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THE protein content of urine and cerebrospinal fluid is commonly determined by the measurement of the volume of protein precipitated by certain reagents. Shevky and Stafford[4] compared previous clinical precipitation methods for protein and developed a more accurate one, using Tsuchiya's reagent. This method has been used and modified by McNaught[1] for cerebrospinal fluid and by MacKay (Peters and Van Slyke[2]) for urine. Samson[3] has presented a micro-precipitation method for serum protein as well as for serum albumin and globulin but does not give figures for the absolute amounts of protein in either case or compare his values with those of other standard methods. In the hope of making available a simple clinical method for blood protein, comparative determinations have been made both by the precipitation and by macro-Kjeldahl methods.

TECHNIC

The procedure adopted was that given for urine by MacKay with the exception that the volume of protein precipitated was compared with the volume of precipitate from a standard casein solution (McNaught). For this standard a solution containing 3.0 gm. casein, 1.0 gm. sodium bicarbonate, 25.0 gm. sodium chloride, and 20 c.c. formalin was made up to a liter and filtered. The nitrogen was then determined by the Kjeldahl method. Such a standard keeps indefinitely at room temperature. The serum was diluted 1 to 50 and 1 to 75 with 2.5 per cent sodium chloride. Four-cubic-centimeter amounts of diluted serum were put in Shevky-Stafford tubes, two tubes being used for the standard, two for the 1-to-50 dilution, and two for the 1-to-75 dilution. The volume of precipitate was thus brought into range with the volume from the standard. Tsuchiya's reagent (1.5 gm. phosphotungstic acid and 5 c.c. concentrated hydrochloric acid made up to 100 c.c. with 95 per cent alcohol) was added up to the 6.5 mark; the tubes were then inverted slowly three times and, after exactly ten minutes, centrifuged for ten minutes at 1,800 revolutions per minute.

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Calculation was made as follows:

$$\frac{\text{Vol. ppt. unknown}}{\text{Vol. ppt. standard}} \times \text{Grams \% protein in standard} \times \text{Dilution of unknown} = \text{Grams \% protein in unknown serum}$$

EXPERIMENTAL RESULTS

Results by the above method are shown in Table I.

The procedure used was adopted as the result of numerous attempts to make the precipitation method accurate for serum. It was found unsatisfactory merely to multiply the volume of precipitate by the dilution and by the factor 0.72, as is done to obtain the amount of protein in urine. Values for serum protein thus obtained vary with the dilution and with uncontrolled changes in the speed of the centrifuge. Therefore a standard protein solution was used. Various modifications, as the use of sodium tungstate precipitation, the use of different standards (horse serum, human serum, albumin, and casein as shown in Table II), a longer period of centrifuging and dilution of the serum and

TABLE I

PRECIPITATION OF SERUM PROTEIN BY TSUCHIYA'S REAGENT

SERUM ANALYZED	SERUM C.C. PPT.*	STAND. C.C. PPT.*	GM. % PROT. IN CASEIN STANDARD	DILUTION OF SERUM	GM. % PROTEIN	GM. % PROTEIN (KJELDAHL)	PERCENTAGE OF ERROR
1. Normal human serum.	0.128	0.126	0.0929	1:75	7.08	7.08	0
	0.120	0.121	0.0929	1:75	6.91	7.08	− 2.4
	0.122	0.120	0.0929	1:75	7.08	7.08	0
2. Normal human serum.	0.160	0.162	0.1394	1:50	6.88	7.06	− 2.5
	0.133	0.129	0.0929	1:74.3	7.11	7.06	+ 0.7
	0.129	0.129	0.0929	1:75	6.96	7.06	− 1.4
	0.114	0.108	0.0929	1:75	7.35	7.06	+ 4.1
	0.115	0.114	0.0929	1:75	7.02	7.06	− 0.6
3. Normal human serum.	0.179	0.205	0.1567	1:50	6.85	6.78	+ 1.0
	0.186	0.200	0.1567	1:50	6.49	6.78	− 4.3
	0.128	0.148	0.0929	1:75	6.03	6.78	−11.1
	0.131	0.147	0.0929	1:75	6.21	6.78	− 8.4
4. Aortic regurgitation. Syphilis.	0.108	0.105	0.0929	1:75	7.18	7.52	− 4.5
	0.117	0.119	0.0943	1:75	6.95	7.52	− 7.6
5. Malignant hypertension. Cardiac asthma.	0.102	0.109	0.0943	1:75	6.61	6.69	− 1.2
6. Hypertension. Syphilitic heart disease. Nephrosclerosis.	0.131	0.106	0.0943	1:50	5.82	6.44	− 9.6
7. Hypertension. Arteriosclerosis.	0.100	0.099	0.0848	1:75	6.42	6.41	+ 0.2
	0.100	0.108	0.0848	1:75	5.89	6.41	− 8.1
	0.105	0.105	0.0848	1:74.6	6.32	6.41	− 1.4
8. Arteriosclerotic heart disease with decompensation.	0.135	0.130	0.0943	1:50	4.89	5.81	− 5.6
9. Arteriosclerotic heart disease. Nephrosclerosis.	0.115	0.125	0.0943	1:50	4.34	4.84	−10.3
	0.123	0.132	0.0943	1:50	4.39	4.84	− 9.3
10. Glomerular nephritis. Pulmonary congestion.	0.095	0.110	0.0943	1:50	4.07	4.47	− 8.9

*Each figure is the average from duplicate tubes.

TABLE II

USE OF DIFFERENT STANDARDS IN THE PRECIPITATION OF SERUM PROTEIN BY TSUCHIYA'S REAGENT

SERUM ANALYZED	STANDARD USED	GM. % PROTEIN	GM. % PROTEIN (KJEL.)	PERCENTAGE OF ERROR
Normal human serum I	Horse serum A	6.94	7.24	− 4.1
	Horse serum A	7.46	7.24	+ 3.1
	Human serum II	6.58	7.24	− 9.1
	Casein A	6.82	7.24	− 5.8
	Casein A	6.92	7.24	− 4.4
Normal human serum II	Human serum I	7.24	6.92	+ 4.6
	Human serum I	7.01	6.92	+ 1.3
Normal human serum III	Human serum IV	7.58	7.61	− 0.4
	Human serum IV	7.84	7.61	+ 3.2
From patient with multiple sclerosis and epilepsy	Casein B	6.69	6.87	− 2.6
	Casein B	7.01	6.87	+ 2.0
	Albumin A	7.39	6.87	+ 7.6
From patient with syphilitic meningitis	Human serum IV	6.87	6.65	+ 3.3
	Human serum IV	7.00	6.65	+ 5.3
	Casein B	6.87	6.65	+ 3.3
	Casein B	5.81	6.65	−12.6
	Albumin A	6.97	6.65	+ 4.8
	Albumin A	6.71	6.65	+ 0.9
From patient with chronic edema	Casein B	3.68	3.85	− 4.4
	Casein B	3.78	3.85	− 1.8
	Albumin A	3.68	3.85	− 4.4

standard with different concentrations of saline have been tried in attempts to make the method more accurate. Results with different concentrations of saline are shown in Table III.

TABLE III

CONCENTRATION OF SALINE	AVERAGE PERCENTAGE ERROR
0.8%	+6.5
1.0%	+4.0
1.5%	+4.1
2.5%	−4.0

In a few cases the precipitates were resuspended in 80 per cent alcohol and recentrifuged. Considerable variation in the volumes of precipitate in duplicate tubes was found in all of these modifications. For example, five volumes of precipitate from the same solution, centrifuged at the same time, varied in one case from 0.175 to 0.190 c.c. of precipitate. In the later determinations it was thus considered necessary to compare duplicate tubes on all samples.

From the tables it may be seen that the majority of the results have an error of less than 5 per cent although they may occasionally vary from the Kjeldahl determination by as much as 12 per cent, especially when the amount of protein is small.

The average percentage error (+6.5 to −4.0 per cent) compares favorably with the error found by Shevky and Stafford[4] for urine (average error, 8.4 per cent; standard deviation, ±9.7 per cent). The results obtained by McNaught[1] on cerebrospinal fluid are, however, somewhat more accurate (1.5 to 5.1 per cent error). It is recognized that an inherent error exists in methods based on the precipitation of colloids, the physical state of which may vary in different ex-

periments. Factors which affect such precipitation have been discussed by Samson.[3] Of these, the ones which have not been controlled in the present determinations are the size and specific gravity of the particles precipitated, the specific gravity and viscosity of the fluid in which precipitation takes place, and to some extent the temperature of the system.

SUMMARY

A simple method for the determination of serum protein is presented and discussed. The volume of protein precipitated from diluted serum by a phosphotungstic acid reagent has been measured in Shevky-Stafford tubes and compared with the volume from a standard protein solution.

With the proper standard at hand, the simplicity and speed of the precipitation method and the small amount of equipment needed, as compared with the Kjeldahl determination, make the precipitation method applicable for clinical use.

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THE REFINING OF ANTISERUMS*

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INTRODUCTION

THE method of refining and concentrating antiserums and antiplasma which is used in this laboratory will be described in this paper. This method is being used for antipneumococcus and antimeningococcus serums and in modified form for other serums. The experimental procedure will first be outlined; then modifications and explanations of the procedure will be given; and finally a brief discussion and summary of the relations will be presented.

EXPERIMENTAL PROCEDURE

The method to be described is that used primarily for refining pneumococcus antiserums.

a. *Preparation of Serum.*†—Immediately after the separation of the serum, 0.5 per cent phenol was added in the form of a solution containing five parts phenol and six parts ether.[1] If this immediate addition was not possible, the phenol was added as soon as practicable. The serum was kept cold. Before beginning the further treatment, the serum was tested as to sterility. If bacteria were present, the serum was filtered through a Berkefeld filter, No. N.

b. *Dialysis of Serum.*—The serum, containing 0.5 per cent phenol, was well mixed, placed in cellophane (No. 600) bags, from 3 to 4 liters in each. To each bag 10 c.c. toluene and 5 c.c. chloroform were added. Each bag was then tied tightly near the top, the top turned over and tied, or an additional piece of cellophane placed over it, in order to eliminate the possibility of contaminants dropping into the bag. Dialysis against running tap water was allowed to proceed for from five to seven days. After forty-eight hours and again after ninety-six hours, 0.2 per cent phenol (in ether) was added to each bag. The temperature of the tap water ranged from 12° to 25° C. at different times of the year. The duration of the dialysis was greater with the cooler water. The sterility of the dialysate was tested after dialysis.

c. *"Acid Protein" Precipitation.*—The dialysates were removed from the bags and mixed, the bags washed with several hundred cubic centimeters of distilled water, which were added to the main bulk. Dry sodium chloride to bring the solution to N/20 was added (2.92 g. per liter). The solution was placed in the ice box overnight. Early the next morning, it was brought to P_H 5.1 with normal acetic acid (γ – dinitrophenol as indicator), allowed to stand in the

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†The serum was obtained from horses immunized with the centrifuged sediment of pneumococcus broth cultures taken up with saline and the vaccine killed with formalin. In general terms, the horses were inoculated three successive days, rested six days, and the series repeated with increasing dosages up to a certain maximum. Test bleedings were taken every two weeks.

cold for from four to five hours, and filtered through paper pulp and "super cel"* on a Buchner funnel until clear.

d. *Preliminary Tests for Antibacterial Substance Precipitation.*—The filtered solution was brought to P_H 5.9 with N NaOH and kept in the ice box. Portions of 50 c.c. were brought to P_H 5.9, 6.1, 6.3, and 6.8 (with N NaOH where necessary) (p-nitrophenol as indicator), diluted with from four to five volumes of Berkefeld filtered tap water, and allowed to stand in the ice box overnight. The next day the mixtures were centrifuged, the precipitates dissolved in one-fifth the volume (10 c.c. each) of 1 per cent sodium chloride solution, and the supernatants were tested for antibacterial substance contents by precipitin or agglutinin reactions (or both.)[2] In estimating the contents in this way, account was, of course, taken of the concentration or dilution of the antibacterial substance in comparison with the original serum.

These tests were carried out under the direction of Miss Georgia Cooper, who outlined the procedure as follows:

"The titrations were made with quantities of antiserum from the following range, which were selected to show the character and the end point of the reaction; 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, 0.001, 0.0005, 0.0002, 0.00001, 0.00005 c.c.

"For the agglutination tests the antiserums were so diluted with physiologic saline that the requisite amounts were contained in 0.5 c.c. Five-tenths cubic centimeter of each of the dilutions and 0.5 c.c. of a fresh eighteen- to twenty-hour broth culture of pneumococci were mixed in test tubes and incubated for two hours at 55° C. Readings were made. The tubes were stored in the ice box overnight and readings again made.

"For the precipitation tests 0.1, 0.05, and 0.02 c.c. of the undiluted antiserums and the smaller quantities diluted to be contained in 0.05 c.c. were obtained with 0.1 c.c. of the precipitating antigens in test tubes. The tubes were rolled for two minutes. Five-tenths cubic centimeter saline solution was then added to each tube and readings were made. The precipitation antigen was prepared by growing pneumococci in phosphate beef-heart broth for about a week, at the end of which the organisms generally were found to be autolyzed. The precipitation antigens before use, when necessary, were centrifuged until clear. With certain exceptions, the end points of precipitation, and of agglutination to a lesser degree, indicated with a moderate degree of accuracy the relative amounts of mouse protective antibacterial substances in the antiserums tested."

If the supernatants showed the presence of appreciable quantities of antibacterial substance (10 per cent or more of the original), in place of procedures "e" and "f" which follow, recourse must be had to procedures "e1" and "f1."

e. *Precipitation of Antibacterial Substance.*—The solution was brought to the desired P_H as found in the preliminary tests, four volumes of Berkefeld filtered tap water containing 0.2 per cent phenol added, and placed in the ice box overnight. The precipitate in general settled well. The bulk of the supernatant was siphoned off, and the rest of the liquid separated by centrifuging.

*Obtained from the Johns-Manville Corporation, New York City.

f. *Solution of Antibacterial Substance.*—The precipitate was dissolved in from one-fifth to one-tenth of the original volume of 0.5 per cent sodium chloride solution containing 0.5 per cent phenol. The amount of solvent was governed in part by the potency of the original material and the potency desired in the final product, and in part by the physical consistency (viscosity) of the material after solution. The solution was placed in the ice box for at least twenty-four hours, then filtered through super cel and paper pulp to remove separated fibrin. One per cent additional sodium chloride (solid) was added and the solution stored at low temperature.

g. *Preparation of Antibacterial Solution for Clinical Use.*—The cold solution was allowed to warm to room temperature and filtered on a Buchner funnel through paper pulp and super cel and then through a Berkefeld filter. If filtration was slow through a fine Berkefeld, preliminary filtration through a coarser (V), followed by filtration through the finer (N), was used. The filtrate was tested for sterility and then bottled in 10, 15, or 25 c.c. vials, as desired. Suitable sterility tests on the vial contents were also carried out. The preparations were kept in the ice box. At times, solid material (presumably protein) separated in the vials at the low temperature but dissolved readily again at room temperature. Since the material is warmed at 37° C. before use clinically, this separation occasions no difficulty. Stock was labelled to be returned in six months. If the solutions became cloudy, they were refiltered and rebottled, as well as retested for potency. Potency tests, either mouse or agglutination and precipitation, were carried out as described elsewhere both on the filtered bulk and on the bottled materials.

e1. *Alternative Method of Precipitation of Antibacterial Substance.*—If the supernatants of the preliminary precipitation tests "d" showed appreciable quantities (10 per cent or more) of the original antibacterial substance, the following method was used. An equal volume of saturated ammonium sulphate solution was added with thorough stirring (so-called 50 per cent saturation), allowed to stand from four to five hours, and filtered on a number of large fluted papers. This filtration was complete overnight.

f1. *Solution of Antibacterial Substance.*—The precipitate was removed from the filters, pressed between sheets of filter paper in a press for some hours, placed in a cellophane bag containing 10 c.c. chloroform, and suitably tied and dialyzed against running tap water for from three to four days. To the solution after removal from the bag, 1.5 per cent sodium chloride and 0.5 per cent phenol (as phenol-ether mixture) were added. The volume was then from one-fourth to one-half of the volume before precipitation. The solution was placed in the ice box for at least a day and then filtered through paper pulp and super cel to remove separated fibrin. Further treatment followed that described in section "g."

NOTES ON THE EXPERIMENTAL PROCEDURE

A. The method outlined is given as applicable to antiserums. Essentially the same procedure can be used with plasma. The greater amounts of fibrin (and fibrinogen) in the latter may increase the acid, perhaps other precipitates, and may also make filtration slower and more difficult. An essential feature of

the method consists in keeping the material as free from bacterial growth as possible. The importance of this fact and the desirability of having no products of bacterial growth present in the final products was recognized early and strongly emphasized, among others by Anderson[2] in 1909 and by Avery and his coworkers[3] in 1917.

B. Dialysis of the serum was an essential feature of the Banzhaf-Klein procedure.[4] This procedure was stated to depend upon the observations of Avery[5] and of Felton[6] that pneumococcus antibacterial substances were precipitated from serums by dilution with distilled water. Dialysis in the Banzhaf-Klein procedure was assumed to remove the salts and so play the part that was taken by dilution. It may be stated that, as a result of dialysis and the accompanying removal of salts, partial, but not complete precipitation of antibacterial substance occurred. The function of the dialysis did not, therefore, appear to be altogether simple. In addition to removing the salts, it evidently involved changes in the character of the globulins. Such changes have been described by Svedberg,[7, 8] who, as a result of molecular weight determinations, considered that one globulin was present in serum initially and that different treatments broke it down into fractions designated pseudoglobulin and euglobulin, and by Reiner and Reiner,[9] who showed that repeated precipitations resolved a globulin precipitable at a definite P_H value (from 5.5 to 6.0), into two globulins precipitable at two different P_H values (5.1 and 6.7). Dialysis evidently caused changes in the natures of the protein fractions which resulted in the subsequent procedures furnishing an immunologically useful product. It was found possible to keep the contents of the bags sterile throughout the dialysis by using the precautions given, although tests* showed that about 70 per cent of the phenol dialyzed out of the bags within the first three days. If the cellophane bags were used for more than one dialysis, they were washed thoroughly and kept in 5 per cent phenol solution until used again.

C. The "acid protein" precipitation as developed by Felton[10, 11] was an essential part of the method. With Types I and II pneumococcus antiserums it was permissible to allow the precipitate in the solution to stand overnight in the ice box; with other antiserums the time should be limited to five hours because of possible inactivation due to the acid condition.

D. The preliminary tests for the best conditions for the precipitation of antibacterial substance were very essential. It was naturally desirable to obtain these conditions in as short a time as possible. Agglutinin and precipitin tests were used, therefore, and not mouse protection potencies. Comparative results were sufficient for the purposes in view, namely, possible losses of material as shown in the tests on the supernatants. A large number of pneumococcus (as well as meningococcus and other) antiserums were tested, but no regularities observed as to the most suitable hydrogen ion concentration. The two best values were found to be P_H 5.9 – 6.1 and P_H 6.8. Apparently a number of variables, including the organism and method used in inoculating the horses, the condition and state of the horses (whether they had been bled over a long or short period of time), the keeping and handling of the serums, etc., all played a part

*Carried out by Edward Buxbaum.

in determining the properties of the protein substances present. These conditions, at present, are not controllable. In this connection, attention may be directed again to the work of Reiner and Reiner referred to earlier in this paper. The classification of proteins to be applied to these different precipitates is not altogether clear. The "acid precipitate" at P_H 5.1, is in part fibrin and possibly euglobulin; the antibacterial substance precipitate may be considered to be pseudoglobulin, since practically all anti substances have been found to be associated with this protein fraction. However, this precipitate did not include all of the pseudoglobulin present in the serum. At times, antibacterial substance was found in the supernatant from such a precipitate. In such a case, recourse was had to precipitation with 50 per cent saturation of ammonium sulphate, the customary method of separating pseudoglobulin.

With the pneumococcus antiserums, it was found a number of times that Types V and VII showed considerable quantities of antibacterial substance in the supernatants. However, this was not always typical of these types, but more probably due to the previous history of the horses in these special cases because serums of these types from different horses caused no difficulty.

The usual methods of carrying out the agglutination and precipitation tests were used. When practicable, both were employed.

E. In this manipulation it was evidently impossible to avoid contamination. Using 0.2 per cent phenol and working in the cold, it was found that bacterial growth was practically negligible or very small and the formation of appreciable quantities of products of such growth avoided.

F. With some preparations, after solution, a gradual separation of protein substance occurred. This may have been fibrin or a conversion product of the globulin. Before use, this cloud or suspension was removed. As a rule, it contained no appreciable amount of active material.

G. The question of sterility was predominatingly important here. The comparatively large concentration of protein present in the final product and the occasional difficulty of filtering through a Berkefeld filter, necessitated special care for this part of the procedure. After various series of tests, it was decided to use 0.5 per cent phenol in all cases although recent experiments, as yet unpublished, by Miss Rosenstein and her coworkers in this laboratory have thrown considerable light on the efficacy of various disinfectants with different contaminants. Because of the possibility of the solutions becoming permanently cloudy on standing, a time limit of six months was placed on the clinical use of the materials. If, upon expiration of this time, the solutions were clear and showed no marked decrease in potency, they could be used directly. If, however, a cloud or suspension was present which did not dissolve on warming to 37° C., the preparation was pooled, refiltered, rebottled, and retested for sterility and potency.

Potency tests have been described and discussed in detail at various times. Agglutinin and precipitin tests were carried out by Miss Cooper essentially as described in "d" and "e." Mouse protection tests were also carried out under the direction and supervision of Miss Cooper. The details of the method were given at various times by Felton,[10, 11] Park and Cooper,[12, 13] Falk, McGuire,

Valentine and Whitney,[14] and perhaps in most satisfactory form by Beard and Clapp.[15]

E1. The necessity of precipitating the total pseudoglobulin fraction by 50 per cent saturation with ammonium sulphate was obvious. Because of the possible inactivation by the salt, the precipitate was filtered on the day it was formed.

F1. The danger of contamination was present in this part of the procedure. The presence of the chloroform, care in closing the cellophane bag, and rapid working when the material was exposed, however, limited bacterial growth.

DISCUSSION

The procedure described is essentially that proposed by Banzhaf and Klein,[4] but with a number of modifications. The "acid protein" precipitation developed by Felton[10, 11] is one of the most important single steps in the method. If properly carried out, this step is fairly dependable in removing chill-producing substances from the preparations. Another important feature of the method is the prevention of bacterial growth and the formation of products of such growth which, if present, may well accompany the antibacterial substances in the various manipulations and produce undesirable clinical reactions.

The method as described was developed with antibacterial substances obtained by the use of the different types of pneumococcus. It has, however, been used in the refining of other antiserums as will be outlined presently. The method has been in use for over two years at the Laboratories of the Department of Health with generally satisfactory results as far as the evidence available indicates. This does not mean that methods used in other laboratories such as various salt precipitations, or the alcohol method of Felton[16] are not equally satisfactory. The method described here is preferred by the writers because of its comparative simplicity.

The chill-producing and possibly other deleterious substances which are occasionally present offer the most direct problem for study. Felton's "acid protein" precipitation method is the most useful process available for eliminating such substances. With some serums one refinement is not sufficient to remove all of these substances, and the process must be repeated (including dialysis for several days, etc.). It is evident that where large amounts of antiserum, such as for pneumococcus Types I and II, are available, it is possible to carry out two, or even more, refinements with profit. With the other pneumococcus types where much less antiserum is available, the losses in refining comparatively small amounts, while in absolute quantity perhaps much the same as with large amounts, are so much greater in relative or percentage figures, that more than one refinement is inadvisable. This is perhaps the reason for the somewhat greater number of reactions observed clinically with the less common types of pneumococcus antiserum preparations, and not the fact that one process of refining is better than another, or that more information is available about certain types.

At the same time, much remains to be done in studying antiserums for the various types. For example, as stated before, in carrying out this work, it was

found that certain preparations of Type V and Type VII pneumococcus antiserums could not be refined by the simple dilution procedure, but required ammonium sulphate for precipitation. Similar materials from different horses could, however, be so refined. The cause for the different behavior is, at present, unknown. All that can be said is that the antibacterial substances were present in different fractions of the "pseudoglobulin" in these cases and that they existed in all probability in different fractions of the protein material in the serums of the horses. Such differences may well be due to repeated and long-continued inoculations and bleedings of the horses.

In connection with the method described in this paper, several facts may be pointed out. The conditions under which the experiments were performed were not satisfactory so far as the possibility of maintaining sterility was concerned. Even under these unavoidably adverse conditions, it was possible in most of the experiments to maintain sterility in the dialyzed solutions. The chill reactions of some of the final products may have been caused by the bacterial contaminations and growths which took place in some of the refinings. But the fact may be stated with some degree of emphasis that, in the opinion of the writers, it is not only possible but feasible to maintain sterility, or at worst to inhibit bacterial growth, under suitable conditions of working. It may be stated again that the method of refining and concentrating antiserums here described is in no sense complete and final. It marks a step in that a method is presented, simple to carry out, and generally satisfactory although the preparations may sometimes require a second or even third reworking.

The questions of determining potencies and the clinical efficacies of the refined preparations do not come within the scope of this paper except in so far as the satisfactory attainment of certain standards is a necessary condition for the use of the method. The study of potency by mouse tests has been given by various workers a number of times. Where sufficiently virulent cultures were available, mouse units were used; otherwise agglutination and precipitation tests were resorted to as measures of the degree of concentration of the antibacterial substances in the refining process. Obviously the final volume of a preparation as compared to the initial volume controls the degree of the concentration but it may be said in general terms that the antibacterial substances were concentrated between five and ten times in the final preparation. The losses in active materials were difficult to estimate because of the inaccuracies inherent in the experimental method of determining potencies. In order to give a figure, which, however, cannot be considered as of a high order of accuracy, it may be said that, as a rule, between 50 and 100 per cent of the active material was recovered.

The description of the method referred to pneumococcus antiserums. The same method, with practically no changes, was used with meningococcus antiserums with equal success. It may also be used for additional antibacterial substances. Its possible application to other materials may be of interest. For instance, some experiments were carried out with scarlet fever antiserum which contained both antitoxin and antibacterial substances. Following dialysis and "acid protein" precipitation, the antibacterial substances were separated by

dilution at the most suitable hydrogen ion concentration followed by the precipitation of the antitoxin with ammonium sulphate to 50 per cent saturation. The clinical results with these two preparations (or more properly, protein fractions) were too few to warrant any statement, but the method is suggestive and may prove of interest and value.

The work described treats of the practical features of refining and concentrating antibacterial substances. The more purely scientific aspects of the problem have not been considered. There is, in fact, no evidence as to the nature of the active groupings or constituents of the anti substances. It would seem as if the time were ripe for a more intensive study of the chemical natures of these physiologically active materials. They, including both antitoxins and antibacterial substances, belong to the protein group of pseudoglobulins, which in general terms are less soluble than albumins and more soluble than euglobulins. This classification of the proteins is still arbitrary and based upon differences in solubility and not, as far as known, upon definite differences in chemical compositions and structures. The work of Svedberg and of the Reiners, to which reference has been made, brings out the arbitrary character of the classifications. The change in solubility of certain constituents on treatments in refining indicates this fact. For example, heating diphtheria and tetanus antiserums with ammonium sulphate to 30 per cent saturation for an hour at 58° C. converts some of the inactive pseudoglobulin into insoluble protein.[17, 18] On standing (or on dialysis), precipitates may be formed with antibacterial products. These phenomena appear to involve the conversion of "pseudoglobulin" into "euglobulin." However, when the arbitrary nature of the classification is considered, to speak of the conversion of such constituents means nothing. Part of a protein fraction on simple treatment becomes insoluble (perhaps in a different nomenclature, denatured), and that is all that is known. Such simple changes taken in connection with the physiologic actions, however, may offer a promising field for study. Similarly, the precipitation of antibacterial substances, best at fairly definite hydrogen ion concentrations, these concentrations differing somewhat with different preparations, would also repay study. So far, chemical analyses have not succeeded in throwing light on any of these phenomena.

SUMMARY

The methods in use at the Bureau of Laboratories of the Department of Health of New York City for refining bacterial antiserums are described. Various applications of these methods are discussed and some general theoretical relations given.

The writers wish to thank Messrs. Jerome Peltz, Eugene Cardone, Albert Glaubiger, and Edward Buxbaum for their aid in carrying out the experiments and in testing the details of the various procedures. Thanks are also due Miss Cooper, Miss Rosenstein, and their assistants for doing the many potency and sterility tests upon which the procedure was based.

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QUANTITATIVE DETERMINATION OF CHLORAL HYDRATE IN BLOOD AND URINE*

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GETTLER and Blume[1] have described a method based on the work of Cole[2] for the quantitative determination of small amounts of chloroform in tissues. The final step in the method is a pyridine color reaction with chloroform, known as the Fujiwara[3] color reaction. This color change is produced not only by chloroform but by all R-C-$halogen_3$ compounds.

In the course of some work we were doing on anesthetics, we found it necessary to determine the quantity of chloral hydrate in the blood and urine, and accordingly, after consultation with Dr. Gettler, we employed his method for this purpose. We met with difficulties, but these were overcome by certain modifications in the method. With these, we were able to determine quantitatively amounts of chloral in solutions containing as little as 0.001 per cent.

The procedure we employ for determining the amount of chloral hydrate

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in blood is as follows: In a thin-walled test tube, about 200 by 20 mm. in size, is placed 2 c.c. of a 40 per cent NaOH solution, to which is added 1 c.c. pyridine (colorless) and then 4 c.c. blood filtrate prepared by the Folin-Wu method. In a similar tube is placed the NaOH and pyridine and 4 c.c. of a solution of chloral hydrate, for a standard. In our work the standard chloral hydrate solutions ranged from 1:20,000 to 1:100,000, the solution finally used for each determination being approximately that corresponding in chloral content to the blood filtrate. The two tubes are shaken simultaneously and equally and kept in a boiling water-bath for exactly one minute. They are then cooled by placing them in an ice bath for one minute. Six cubic centimeters of water is then added to each and the contents mixed by inversion. The contents are then filtered into colorimeter cups and readings made in the usual manner. The readings should be made within ten minutes from the time of heating, as the color changes on standing longer.

For determining the chloral in the urine the same procedure is followed but here the urinary pigments may interfere with the colorimetric reading. To obviate this instead of the final dilution with 6 c.c. of water, part of the water is replaced by an amount of urine corresponding to that taken for the chloral determination. Since the pyridine reaction does not occur in the cold, samples from the same urine are used in the water replacement. It is advisable that the concentration of the unknown should range between 0.01 and 0.05 mg. per c.c. If it is below 0.01 mg. it is necessary to add known quantities of chloral to obtain a satisfactory reading.

In checking the accuracy of the method described for blood chloral concentration, quantities of chloral ranging from 0.25 to 1 mg. were added to 3 c.c. of blood and the color read against standard aqueous solutions. The blood figures were within ± 3 per cent of the standard.

It should be pointed out that the method used seems to apply only to free chloral, or at most chloral which has been very little modified. When conjugated with glucuronic acid, chloral does not develop the pyridine color.

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A METHOD OF DAMPING THE KROGH TYPE OF SPIROMETER FOR STUDYING THE RESPIRATION OF RABBITS*

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WHILE studying the effects of morphine and its derivatives on the respiration of rabbits the author had reason to believe that the Krogh spirometer being used did not follow accurately the respiratory volumes of the animals. The apparatus consisted of a 40-liter rebreathing tank connected directly to a

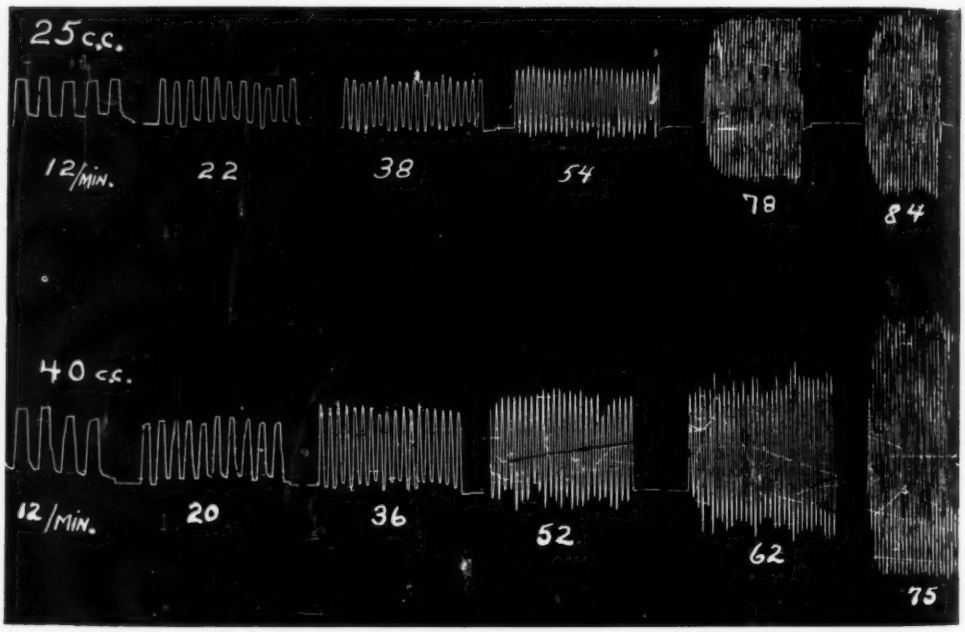

Fig. 1.—A record of the variations in amplitude of excursion of the undamped Krogh spirometer as described in the text. Upper tracing shows the variation at a volume of 25 c.c. and the rates (per minute) indicated. The lower tracing was made with a volume of 40 c.c. at the rates indicated.

Krogh spirometer (500 c.c. capacity) that recorded over two pulleys on a smoked kymograph paper. The float of the spirometer was made of phosphor bronze (0.038 cm. thick), and required a counterbalance weight of 80 gm. at a distance of 10 cm. from the axis of rotation. As a test of the ability of such a system to follow the respiratory volumes encountered, a 50 c.c. syringe was used to introduce known volumes of air into and out of the rebreathing tank. A kymograph record of the excursions of the spirometer showed at once that

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overshooting occurred at high rates (Fig. 1). The extent of this overshoot is shown by plotting the millimeter excursion of the spirometer against the rate at different volumes as given in Fig. 2. This graph shows an enormous overshoot starting at about 30 respirations per minute and a slight undershoot at rates below this point. It is obvious that with such a recording system no accurate

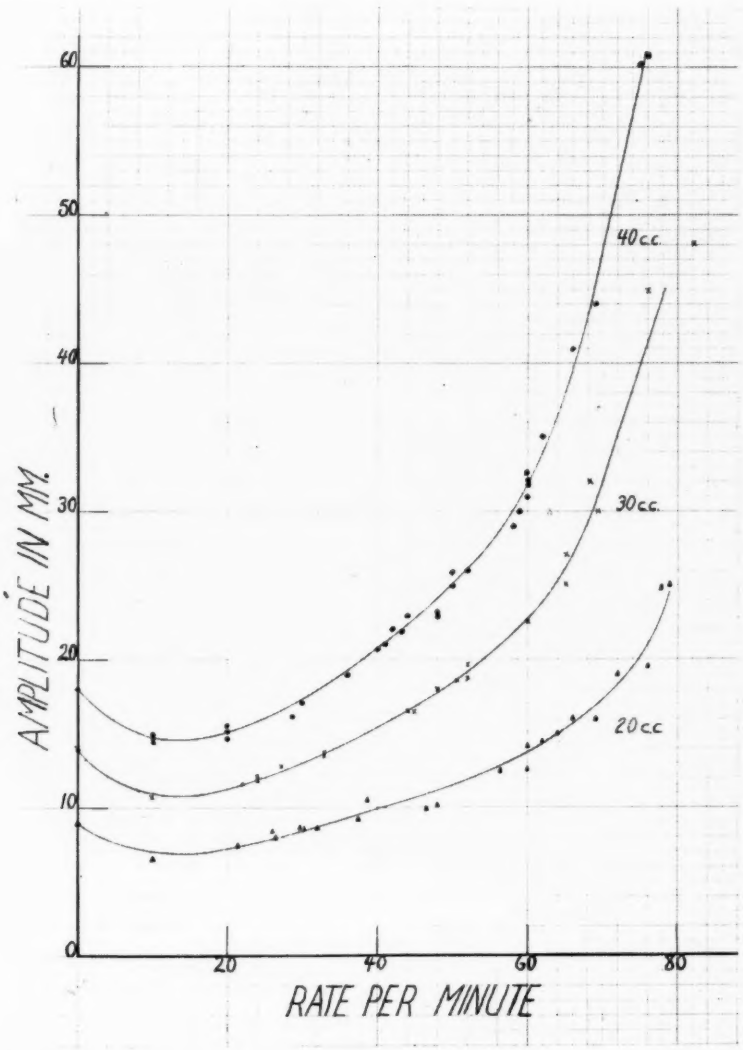

Fig. 2.—The variation in amplitude of excursion of the undamped Krogh spirometer with rate. Each curve represents a constant volume as indicated.

determination of respiratory volume can be made on rabbits since the respiratory rate of these animals varies between 30 and 80 per minute. Especially is this true if the animal is forced to breathe CO_2, which is frequently used as a respiratory stimulant.

A spirometer float of extremely thin (0.035 cm.) aluminum was made in an effort to reduce the mass, and thus the moment of inertia, sufficiently to allow

accurate recording at the rates involved. The overshoot was reduced, but by no means eliminated.

Finally an aluminum damping vane (diameter 5 cm.) was fastened, by

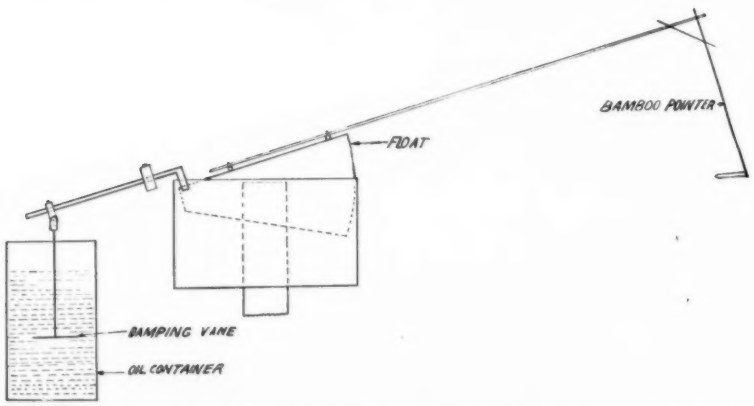

Fig. 3.—Diagram of the Krogh spirometer and the method of damping. The float, counterbalance arm, damping vane, and attachment are made of aluminum.

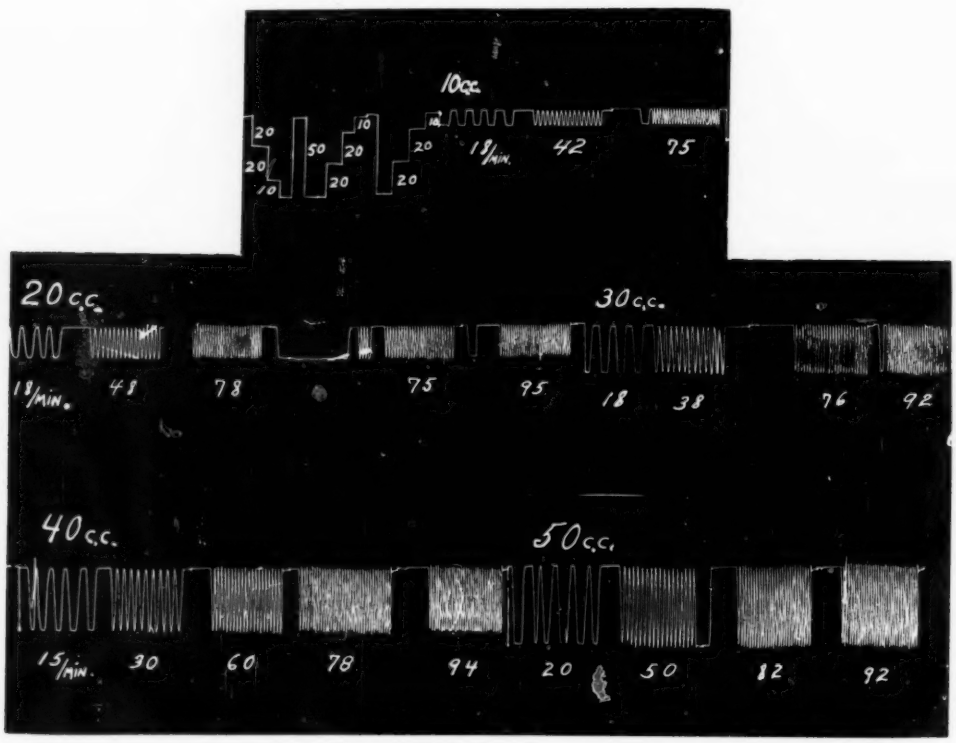

Fig. 4.—A record of the amplitude of excursion of the damped Krogh spirometer at the volumes and rates (per minute) indicated.

means of a hinged joint, to the counterbalance arm as shown in Fig. 3. This vane, immersed in oil as shown, effectively damped out all variations in the excursion of the spirometer at rates up to 100 per minute. The oil used is com-

mercial motor oil of a viscosity usually designated by S.A.E. 40. This oil critically damps the apparatus here described at room temperatures between 22° C. and 26° C.

A test of the pressures developed in the rebreathing tank showed that no significant change in pressure was introduced by the use of this damping vane.

Fig. 4 shows a record made by introducing volumes from 10 c.c. up to 50 c.c. at the rates indicated using the damped spirometer. As can be seen, there is no variation in depth with rate, and a plot of the excursion of the pointer on the spirometer against frequency at any one volume gives a straight line, parallel to the abscissa, up to frequencies of 100 or more.

COMPARISON OF THE KOLMER-WASSERMANN, KAHN, AND ROSENTHAL TESTS FOR SYPHILIS*

With Special Reference to the Use of the Latter in the Public Health Laboratory

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IN RECENT years there has been a growing tendency on the part of syphilologists and laboratorians not to rely on a single test for the serologic diagnosis of syphilis. Some laboratories have replaced the time-honored Wassermann test with some simpler method, such as the Kahn test, but it is probably safe to say that most laboratories today use some modification of the Wassermann, checking the results by means of one or more of the flocculation tests. Of these the Kahn is probably the best known. The Kline test has more recently come to the fore as a simple microscopic test; it has been recommended by Kolmer[1] as being superior to the Kahn as a check test. In some laboratories it is routine to use both macroflocculation and microflocculation tests to check the Wassermann results. The purpose of this study was to determine the applicability of the newer Rosenthal microscopic test for use as a check method.

In 1929, Rosenthal[2] described a microflocculation method which was technically much simpler than the Kline test in that neither serum nor antigen was diluted, either before or after mixing, and tabulated the results obtained by testing 1,066 serums by the new method and by the Wassermann test. He found that the new test gave more clear-cut reactions with the weakly positive and doubtful Wassermann serums. Since it required only a small quantity of serum, it could also be used where only finger blood was available. In 1932, Tulipan and Director[3] reported the results of the Wassermann and Rosenthal tests on 1,067 serums, mostly syphilitic. They found that the two tests agreed in 986 instances, disagreed in 81. No disagreements were noted in individuals not clinically syphilitic. Of the 81 disagreements on clinically syphilitic cases, the Wassermann was positive and the Rosenthal negative on 17; the Wassermann

*From the City Health Department Laboratory.
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was negative and the Rosenthal positive on 64. The majority of these disagreements occurred in tertiary cases and patients who had received treatment. These authors felt that the Rosenthal test tended to become positive earlier in the disease and to remain positive longer under treatment than the Wassermann. They recommended it for emergency uses, such as testing donors for transfusions, also to rule out negative serums before testing by more complicated methods.

In 1933, Burdon and Duggan[4] reported tests on 1,235 serums by the Kahn and Kline methods; on 400 of these they also used the Hinton, Meinicke clarification, Sachs-Georgi and a modified Rosenthal test. On the basis of their results they did not recommend the Meinicke or Sachs-Georgi methods, while they felt that the Hinton test was inherently limited in its practicability. Concerning the Rosenthal test they stated: "Our brief experience with the Rosenthal test has led us to believe that it is a method of real value as a supplementary procedure and that it would be especially useful in those laboratories where the Kahn test is done routinely, since the Kahn alcoholic extract, the only necessary reagent except a 2 per cent cholesterin in acetone solution, would be on hand at all times. The method is simple, rapid and economical. The results show that it is practically as sensitive as the Kline test, and no false positives were recorded. The reactions, however, were by no means as clear-cut as those of the Kline method, and in tests with normal serums the emulsion may develop a diffuse granularity which one must learn by experience to disregard." The modification of technic described by these authors will be detailed later.

TECHNIC

As devised by Rosenthal, the test was conducted as follows: Capillary pipettes were drawn from glass tubing, being kept together to form matched pairs. With one pipette 4 drops of inactivated serum were blown into the depression in a hollow-ground slide; with the other one drop of the antigen was added to the serum. The two were then mixed with a glass rod and, after a short time, examined under the low power objective. This method was used without significant change by Tulipan and Director. For the present study the modification of Burdon and Duggan was used. By this method the test is carried out as follows:

Glassware: Slides, 3 in. by 2 in. with twelve paraffin rings are substituted for the hollow-ground slides. The paraffin rings are made according to the technic for the Kline test.[5] I have found that a piece of 22 or 24 gauge nichrome wire makes a more sturdy loop than the two turns of iron wire suggested by Kline. Slides with twelve depressions ground in would be a timesaver where large numbers of tests are to be run, but for most laboratories the cost would probably be prohibitive. One eastern supply house quoted such slides at approximately $2.00, each.

Pipettes: Those used for the Wassermann or Kahn tests may be employed.

Antigen: No. I. Kahn standard alcoholic beef heart extract (not cholesterinized).

No. II. Two per cent solution of cholesterin in chemically pure acetone.

The stock solutions are kept at room temperature and mixed in equal parts for use. The mixed antigen keeps well for from one to two weeks. Rosenthal tinted the antigen with methylene blue, but this is not essential to ease in reading.

Method of Test: Five-hundredths cubic centimeters of undiluted inactivated serum is pipetted into the paraffin ring. More than twelve tests should not be set up at one time. A drop of the antigen mixture is then allowed to fall on the surface of each serum from a pipette delivering about 0.015 c.c. per drop. I have found that a Schick test (27 gauge) needle on a small syringe makes a very satisfactory substitute for the capillary pipette, delivering approximately the correct amount of antigen. The annoyance of having the tip of the pipette become broken, possibly in the midst of a series of tests, is also obviated. Magath[6] suggested sealing a needle into a glass tube for use as an antigen pipette for the Kline test; this expedient could also probably be successfully used in the Rosenthal method. The time of addition of antigen to the serums is noted, and each preparation stirred with a separate toothpick. They are then examined under the low power objective, focusing on the surface of the drop. A preliminary observation is made of the character of the granular field; the final reading is made within from five to eight minutes, never more than ten, from the time the antigen was added. A negative reaction appears as a granular suspension, evenly distributed; slight clumping is noted as doubtful; well-defined clumps as positive, and large clumps in a clear field as strongly positive. A clear distinction between 2, 3, and 4 plus cannot be made.

Wassermann and Kahn Tests: Kolmer's three-tube qualitative modification of the Wassermann test[7] was used as the basic test in the examination of all serums, and agreement with it was considered the criterion of efficiency. The Kahn test was performed in accordance with the directions given by Todd and Sanford,[8] using antigens obtained from the Difco Laboratories. Kahn tests on the first 707 serums in the study were made with the "standard" antigen, the remaining 483 were tested with the "sensitized" antigen.

Interpretation of Results: The majority of the blood specimens examined in this laboratory come from the Municipal Venereal Disease Clinic, and of these the largest percentage is from colored patients. Cases of anticomplementary Kolmers accompanied by agreement of the Kahn and Rosenthal tests were considered as absolute agreement. In case of discrepancy in the two latter tests, additional specimens were taken, if necessary after a provocative injection of neoarsphenamine, until either positive or negative Kolmer reactions were obtained. However, these cases of anticomplementary Wassermanns with disagreement in the two check tests are listed as separate classifications in Table I, in which the various combinations of results are tabulated. A doubtful Rosenthal reaction with a negative Kolmer and Kahn test was considered as negative, except in treated cases, but with a positive Kolmer and Kahn reaction it was considered positive. In treated cases a positive or doubtful Rosenthal, even with a negative Kolmer and Kahn test, was considered as indicating the necessity for further treatment.

RESULTS OF TESTS

In this series of tests we have examined 1,185 specimens of blood and 5 of spinal fluid. Four of the latter were negative by all three tests, the fifth gave a 4 plus Kolmer, negative Kahn and doubtful Rosenthal. The results with the Rosenthal test as applied to spinal fluid are thus seen to be rather inconclusive, and further study along this line would be desirable; however, these five specimens are included in Table I, with the results on the 1,185 blood specimens. There were 836 specimens for diagnosis; these are designated "New" in the table. There were 290 for the result of treatment, and 64 were unclassified in this respect. Some of the patients classified as new were known to have received

TABLE I

KOLMER	KAHN	ROSENTHAL	NEW	TREATED	UNCL.	TOTAL
Neg.	Neg.	Neg.	536	146	45	727
Pos.	Pos.	Pos.	192	49	9	250
			728	195	54	977
Pos.	Neg.	Pos.	40	39	4	83
Neg.	Neg.	Pos.	10	23	3	36
Pos.	Neg.	Neg.	14	8	2	24
Neg.	Pos.	Pos.	4	11		15
Neg.	Pos.	Neg.	5	4		9
Pos.	Pos.	Neg.	3	1		4
Ac.*	Neg.	Pos.	2	3		5
Ac.	Pos.	Neg.	2	2		4
			80	91	9	180
Neg.	0†	Neg.	14			14
Neg.	0	0	5	1		6
0	Neg.	Neg.	3	1	1	5
0	Neg.	Pos.	1			1
Pos.	Pos.	0	2			2
Neg.	Neg.	0	2			2
Pos.	0	0	1			1
Ac.	0	Pos.		2		2
			28	4	1	33
Total (all tests)			836	290	64	1,190

*Ac.—Anticomplementary.
†0 – Test not made.

TABLE II

	KOLMER		KAHN		ROSENTHAL	
	NO.	PCT.	NO.	PCT.	NO.	PCT.
Positive	364	30.59	284	23.86	389	32.69
Negative	809	67.99	883	74.20	790	66.39
Anticomplementary	11	0.92				
Not done	6	0.50	23	1.94	11	0.92
Total	1,190	100.00	1,190	100.00	1,190	100.00

treatment from one to twenty years previously, but their blood tests were not made as a check on such treatment, but rather for diagnostic purposes.

The first division of Table I records the tests which may be considered as absolute agreement; the second division those in which disagreements occurred

among the three methods; and the third division those specimens on which one or two of the tests were not done, due usually to an insufficient amount of serum, breakage of tubes, etc.

Consideration of Tables I and II shows that the Rosenthal test is apparently more sensitive than either of the other two, especially in the cases of patients who have received treatment. It will also be noted that the greatest number of discrepancies falls in the class of treated cases. These findings are in accord with those obtained by Tulipan and Director. Contrary to these authors and also to Burdon and Duggan, Table I shows a number of examples of what must have been false positives although several of the serums may have been from early cases of syphilis or from patients who had previously received treatment.

Of the 180 disagreements noted in the second division of Table I, 147 showed negative Kahn tests with positives by either one or both of the other methods. Of this group, 94 were included in the 707 specimens examined with the standard antigen, while 53 were part of the 483 tests made with the sensitized antigen. This gives percentages of 13.2 and 10.9, respectively. This reduced sensitivity of the standard antigen was also remarked by Burdon and Duggan.

From the standpoint of practicability, it is desirable to know what percentage of agreement may be expected from the tests under consideration, or from various combinations of them. These figures are tabulated in Tables II and III.

TABLE III

IN AGREEMENT	NUMBER	PERCENTAGE OF TOTAL
Kolmer, Kahn and Rosenthal	977	82.1
Kolmer and Rosenthal	1,083	91.0
Kolmer and Kahn	1,021	85.7
Kahn and Rosenthal	1,021	85.7

SUMMARY AND CONCLUSIONS

Results are presented covering the examinations on 1,185 specimens of blood serum and 5 spinal fluids by the Kolmer-Wassermann, Kahn, and Rosenthal tests. Comparisons are tabulated, giving the percentages of agreement between the various combinations of tests. On the basis of these results, we may conclude that the Rosenthal test is an accurate, simple, and economical method, agreeing well with the Wassermann and Kahn tests. On these grounds it can be recommended as a check test, or as a preliminary test, to be checked later by other methods. It is especially applicable to the public health laboratory, where the short time requirement (twelve tests may be set up and read in ten minutes) will commend it to most laboratorians. The fact that it may be performed with finger blood and in such a short time makes it a valuable procedure for use in the hospital laboratory as an emergency method, such as for testing donors for transfusions. In this connection it is to be remembered that the antigen may be kept on hand and a single test set up without any bother of making dilutions or waste from preparing a quantity of reagent. It may also

be found useful in the routine suggested by Tulipan and Director, that of testing first by some simple method to rule out the frank negatives, then testing positive and doubtful serums by other methods to confirm or disprove the preliminary findings.

As with any study of this type, the results obtained give rise to other interesting problems which must be passed up in this laboratory. The question of whether the positive Rosenthals with negative Kolmer and Kahn reactions in new patients are really false positives or whether they are incipient syphilis or beginning cerebrospinal syphilis, could be solved by the aid of a well-organized social service follow-up work. It would also be interesting to tabulate such a series as this one on the basis of the part of the body involved, that is, visceral, cerebrospinal, bone, etc. This could best be done in a large hospital center.

The author wishes to express his appreciation to the following men for their criticism of the manuscript during its preparation: Dr. G. D. Johnson, Dr. H. H. Wescott, Dr. K. D. Graves, Dr. W. F. Manley, Dr. W. C. Stephenson, Jr., and Dr. K. T. Redfield.

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AN AUTOMATIC-FILLING INK RECORDING SYSTEM AND PERMANENT TYPE WATER MANOMETER*

J. P. QUIGLEY, PH.D., CLEVELAND, OHIO

AN INK recording system on apparatus such as the water or mercury manometer, signal magnet, etc., has distinct advantages over the ordinary smoked paper recording method. The apparatus indicated in Fig. 1 is sturdy, durable, simple, and of light weight. The ink recorder and the permanent type water manometer (Fig. 2) have been continuously used with satisfaction in balloon studies of gastrointestinal motility for the past two years. A manometer having the dimensions indicated is satisfactory for many purposes, and manom-

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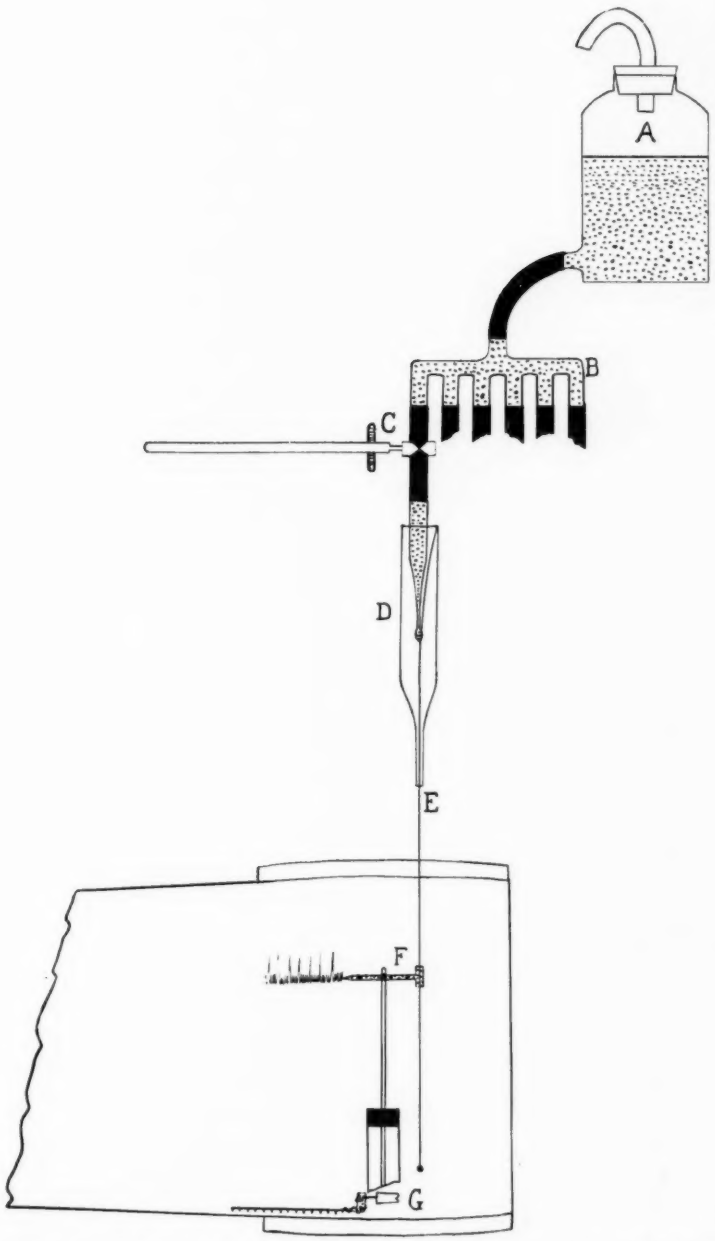

Fig. 1.—Automatic-filling ink recording system. *A*, Ink reservoir; *B*, distributing unit (for group of manometers); *C*, Gaskell clamp compressing rubber tube filled with wicking; *D*, glass tube guide for thread; *E*, double thread of Richardson silk "A" (feeds ink to *F* and carries away excess) weighted with shot; *F*, writing point of thin-walled glass capillary tubing; *G*, cup-type writing point fastened to signal magnet by spiral of spring wire.

eters of this type, which are exact duplicates or definite variations, can be made to subserve special purposes. Difficulties related to air leaks are largely eliminated, and the necessity of making replacements is decreased by the avoidance of most of the rubber tubing employed on ordinary manometers.

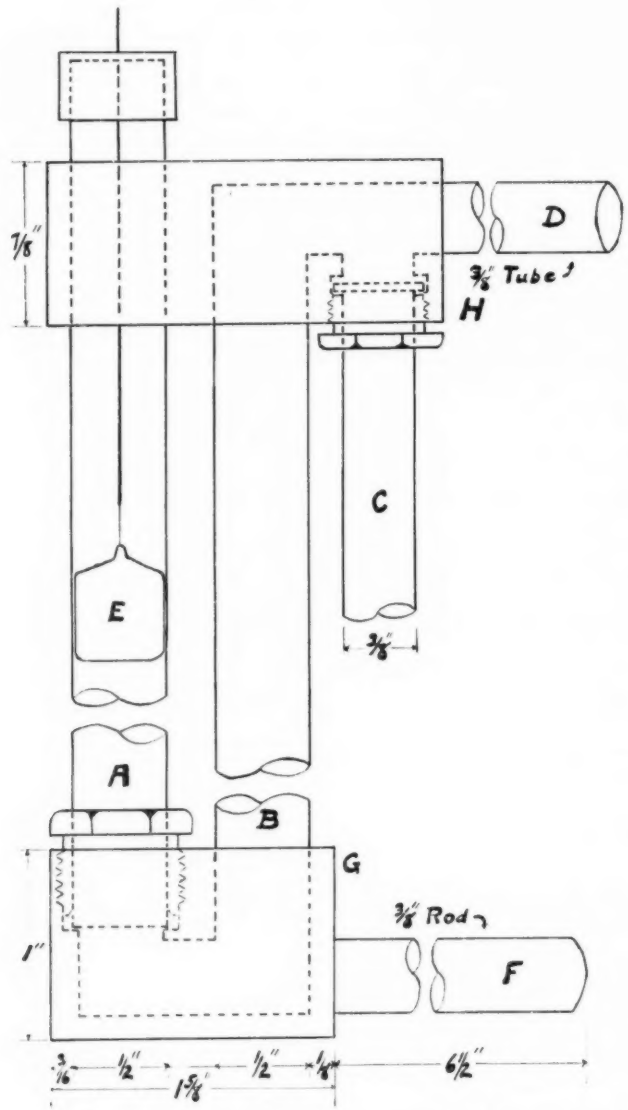

Fig. 2.—Permanent type water manometer. Glass tube *A* and brass tube *B* set into brass blocks, *G* and *H*; *C*, tube of soft copper or lead (reaches to balloon tube inserted in animal); *D*, brass tube sweated into *H* and fitted at outer end with short length of rubber tube and clamp (employed in adjusting pressure within system); *E*, thin-walled glass float sealed to float rod of flexible wire and light weight capillary tubing; *F*, brass rod sweated into *G* (used with *D* to support manometer).

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

UNDULANT FEVER, "Brucellin," a Possible Specific for, Huddleson, I. F., and Johnson, H. W. Am. J. Trop. Med. 13: 485, 1933.

The authors comment upon the various pitfalls surrounding the accurate diagnosis of Brucella infection, emphasizing the uncertainty of blood cultures, especially in infections of the suis or abortus varieties, and the difficulties of accurate interpretation of agglutination titers.

They have developed what they consider a satisfactory procedure for the detection of active Brucella infection utilizing both an allergic skin reaction and a determination of the opsonophagocytic power of the patient's blood.

These procedures are carried out as follows:

Brucella Allergic Skin Test.—The test solution is a soluble nucleoprotein fraction made from the three species of Brucella in 1:1,000 dilution in slightly alkaline physiologic saline.

The test is made by injecting about 0.1 c.c. of the fluid intracutaneously in the upper anterior surface of the forearm using a 26 gauge needle. The size of the local reaction, which is characterized by a circumscribed erythema and edema, may vary from 1 to 2 inches in diameter and appears within from four to forty-eight hours after injection. It may persist for from forty-eight to ninety-six hours. There is invariably no necrosis or sloughing of the tissue at the point of the local reaction. In the infected, the local reaction may be accompanied by a more marked manifestation of symptoms. Those that are hypersensitive will show a severe systemic reaction along with the local reaction. Those that have not been sensitized to Brucella and who are probably susceptible to infection show no local or systemic reaction. One often sees in certain individuals an erythema, about ½ to 1 inch in diameter with no edema around the point of the injection. It has the appearance of a nonspecific reaction.

Determination of Opsonophagocytic Power of Blood.—(See abstract published in June issue, page 1024: Huddleson, Johnson, and Hamann, "A Study of the Opsonocytophagic Power of the Blood and Allergic Skin Reaction in Brucella Infection.") This is a modification of the Leishman-Veitch technic and consists in mixing equal quantities of a citrated (0.8 per cent sodium citrate) fresh blood and a heavy bacterial suspension of living organisms in a small Wassermann tube, incubating at 37° C. for thirty minutes, and subsequently making spreads and staining with Hastings' stain. The addition of a definite amount of sodium citrate prevents clotting of the blood and inhibits the action of Brucella opsonins which are present in the serum of many normal individuals.

This paper is particularly concerned with a report of the therapeutic results obtained with "Brucellin," a product developed by the authors which may possibly prove a specific for the treatment of undulant fever.

This agent is prepared by growing each of the three species of Brucella in separate flasks of beef liver infusion broth, adjusted to a P_H of 6.6, for sixty days at 37° C. The inoculated flasks are shaken vigorously at weekly intervals. At the end of the stated growing period, each flask is examined bacteriologically and, if found contaminated, is discarded. The broth is clarified by centrifugation at a speed of 3,000 r.p.m. for four hours. The clear liquid is decanted and adjusted to a P_H of 7 with N/1 HCl. The product from each of the three species is now pooled and filtered twice through sterile Berkefeld N filters. The filtered liquid is transferred to sterile flasks or tubes and incubated for five days at 37° C. to determine sterility. It is standardized and transferred to sterile 1 c.c. vials or stored in larger containers until ready for use. The duration of its potency has not been fully determined.

When a certain diagnosis of undulant fever has been made, an intradermal injection of about 0.05 to 0.1 c.c. of "Brucellin" should first be given to determine the sensitiveness of the patient to this material. If no marked systemic reaction is elicited within forty-eight hours after the intradermal injection, one may give 1 c.c. intramuscularly. This dose should be repeated at intervals of three days until four injections are given. The intramuscular injections usually cause a local reaction and the temperature of the patient to rise to 104 or 105° F. About one to three hours after the temperature reaches its highest point, it begins to fall by steps or may rapidly drop to near normal. There is as a rule an increase in the severity of the symptoms, such as headache, muscular pains, chills, and sweating. The increase in the severity of the symptoms usually does not persist for more than twenty-four hours. The temperatures of patients treated within the fourth week after the onset tend to return to normal or subnormal after the second injection.

If the systemic reaction from the intradermal injection is quite severe, it is advisable to start the intramuscular injections with 0.1 or 0.2 c.c. If there is no severe systemic reaction following the intramuscular injection of the smaller amount, the succeeding dose at the end of the three days may be doubled. Each dose thereafter may be doubled, providing the systemic reaction is not severe, until 1 c.c. is reached. Three successive 1 c.c. injections may then be given. Certain individuals show very little systemic reaction to 1 c.c. amounts of "Brucellin." Such patients do not respond to treatment until after the dose is increased to 3 or 5 c.c. amounts.

In case children are treated with "Brucellin," the beginning dose should be 0.2 c.c. If the reaction which follows is not severe, each succeeding dose at three-day intervals may be doubled until 1 c.c. is reached. Three successive 1 c.c. injections may then be given.

The ambulatory or chronic type of case of long standing does not respond as readily to four 1 c.c. injections of "Brucellin" as those of three months' duration and less. It may be necessary to gradually increase the dose to 5 c.c. by 1 c.c. steps in order to effect recovery in cases of long standing. Several cases, however, of from eight to twelve months' duration have responded very readily to four injections of "Brucellin." The response to treatment made by infected infants and children, including those in which the disease has assumed the meningeal form, is very striking.

One should not try to avoid systemic reactions from injections of this material. It is necessary to produce two or three systemic reactions in order to bring about recovery.

If it is possible, one should determine the opsonocytophagic activity of whole citrated blood for Brucella after the fourth injection as it has been observed that the phagocytic activity of the blood for Brucella is one of the surest criteria of complete recovery from infection. In the absence of such a test one must be guided in determining recovery by the disappearance of the clinical manifestations of the disease, by the appearance of a subnormal temperature, change in mental attitude of the patient and marked increase in the appetite.

Because of the danger of severe systemic reactions as a result of sensitization, it is essential that "Brucellin" be used only for active cases and never injected into a recovered patient. The necessity for accurate distinction between a recovered patient—recognized by the presence of agglutinins, for example—and an active infection is obvious and cannot be overemphasized.

"Brucellin" has been used in the treatment of 80 cases with excellent results.

SWIMMING POOLS, Some Factors Involved in the Use of Chloramines for the Disinfection of, Fletcher, A. H., and Link, E. C. Am. J. Pub. Health 23: 1255, 1933.

The decided advantage in the use of chloramines for disinfection of swimming pools having a slow rate of recirculation, poor spacing of inlets, hand application of chlorine, or those operated as fill-and-draw pools, and especially when the pool is out of doors, has prompted its use for another season.

The use of chloramines instead of chlorine alone requires certain extra tests and control to guard against its limitations. Some of these are: (a) the requirement of higher

residuals, that is from 0.7 to 1 p.p.m.; (b) the control of the ratio of ammonia to chlorine and of the free ammonia content in the water; (c) the control of the P_H of the water routinely; and (d) the collection of all samples in sodium thiosulphate-treated bottles.

The duplicate series of tests on ordinarily collected samples and dechlorinated collected samples showed a lag in the killing power of chloramines over the chlorine on *B. coli.*

A moderate excess of sodium thiosulphate when used as an antichlor was found to have no disinfecting effect.

Copper sulphate, if added every second night after closing at a dosage rate of 5 lb. to each 100,000 gal. of water, definitely and completely controlled algae or slime growth at any pool where it occurred in Memphis.

STREPTOCOCCI, Hemolytic Properties of the Mastitis, Hansen, P. A., Hucker, G. J., and Snyder, M. A. Am. J. Pub. Health 23: 1262, 1933.

Of 91 authentic strains of *S. mastitidis* being carried in various laboratories as typical streptococci associated with mastitis 50 were of the alpha and 41 of the alpha prime hemolytic types.

The streptococcus commonly associated with chronic or subclinical mastitis always produces the alpha or alpha prime type of hemolysis on blood agar, bordering on a slight beta hemolysis. Strong beta hemolytic strains were not found among the cultures. Two strains of streptococci from mastitis were beta hemolytic, but they were found by cultural studies to be different from the type generally found associated with mastitis.

A careful study of the authentic mastitis streptococci isolated from chronic cases reported to be beta hemolytic were found to be of the alpha prime type. This distinction is apparent when deep colonies are examined by a stereoscopic binocular microscope.

Blood from sheep, ox, and horse was used with similar results. The type of blood did not appear to affect the type of hemolysis produced on blood plates.

PREGNANCY, The Sex Determination Test of Dorn and Sugarman, Curphey, T. J., and Romer, A. S. J. A. M. A. 101: 1680, 1933.

From their experimental study the authors conclude: It is evident that there is no constant agreement whereby the changes excited in the testicle of the rabbit can be used to predict the sex of the unborn child.

Using a pure bred strain of New Zealand white rabbits, they were unable to confirm the observations of Dorn and Sugarman as to the prediction of sex in the unborn child.

The age of the experimental animal and the anatomic location of the testicle at the time of intravenous injection of urine of pregnancy apparently plays no part in the matter of testicular stimulation.

There is present in the urine of pregnant women a so-called spermatogenic factor unassociated with the sex of the unborn child.

The need exists for a further investi[illegible]ion as to a possible relationship between this spermatogenic factor and toxemic states [illegible]nancy.

TUBERCULOSIS, Effect of Vacci[illegible] With BCG on Children From Tuberculous Families, Park, W. H., Kereszt[illegible] [illegible]ishulow, L. J. A. M. A. 101: 1619, 1933.

From the resul[illegible] [illegible]ge ions it appears that B. C. G. is so attenuated that even under the most [illegible] of artificial cultivation it is difficult to increase its virulence to any de[illegible] five years, the virulence of the B. C. G. vaccine was tested on 165 rabi[illegible] gs but no evidence was found of any increase in virulence. Twenty [illegible] id-fas. bacilli that were recovered from the cold abscesses of sevent[illegible] [illegible] one to six months' stay in the body showed no increase of virulence, [illegible] B. C. G. in colony morphology and were nonvirulent for rabbits an[illegible]

One culture was recovered from the mesentery of an orally vaccinated child six weeks after vaccination. This culture showed no increased virulence and culturally was typically like B. C. G.

The pathologic material that was obtained from children who were vaccinated with B. C. G. and died of other infections showed no evidence that B. C. G. tended to increase in virulence during its residence in the human body.

On the basis of their limited experience and on the basis of publications of others, the authors do not think that slight primary tuberculous infection acquired by natural infection or produced by vaccination diminishes resistance against future superinfections by tuberculosis.

Children of tuberculous families vaccinated orally with B. C. G. show lower mortality from tuberculosis than corresponding controls.

None of the parenterally vaccinated children died of tuberculosis, whereas the children who were not vaccinated and were similarly exposed showed about 3 per cent mortality from tuberculosis. There was no appreciable difference in the mortality of the controls who had negative roentgen findings in the chest when they were first seen, whether they had negative or positive initial tuberculin tests.

Tuberculin tests performed on 148 nonvaccinated children followed up since birth and on 205 children orally vaccinated with B. C. G. before their tenth day showed, during the first two years of life, from 20 to 40 per cent more positive Mantoux tests among the latter cases. The allergy produced by B. C. G. apparently does not usually last for more than two or three years.

Comparatively few of the control children developed positive reactions to tuberculin, even when they were more or less exposed to open tuberculosis, i.e., 20 per cent in the first year and 50 per cent up to the fourth year.

If repeated tuberculin tests are performed through the years, a small percentage of the slightly or moderately positive reactions become negative.

NEPHRITIS, Types of and Their Management, Christian, H. A. J. A. M. A. **102**: 169, 1934.

The present study of lipoid nephrosis and the so-called "nephrotic syndrome" suggests that lipoid nephrosis, in adults at least, is a form of Bright's disease.

The frequent association of lipoid nephrosis with definite glomerular nephritis, the fact that cases of clinically pure lipoid nephrosis may terminate in uremia from the end-stages of glomerular nephritis, and the fact that not a single case typical of lipoid nephrosis in an adult has come to postmortem examination at the Mayo Clinic suggest that at least most cases of lipoid nephrosis represent a stage in, or an unusual type of, glomerular nephritis. If other cases occur, they are extremely rare.

The relatively high percentage of patients who have been cured of lipoid nephrosis, or who have shown marked and prolonged improvement, and the relatively slight evidence of glomerular nephritis in most instances at postmortem examination, even among patients who have given clinical evidence of glomerular nephritis, justify the grouping of these patients clinically in a separate group from that of ordinary glomerular nephritis. As long as the term lipoid nephrosis has been so uniformly accepted there seems to be no good reason for changing it.

SERUM PROTEINS, In Diseases not Primarily Affecting the Cardiovascular System or Kidneys, Peters, J. P., and Eisenman, A. J. Am. J. M. Sc. **186**: 808, 1933.

From an analysis of 109 total protein determinations from 52 individuals and of 50 protein fractions from 34 individuals, the following limits of normal variation have been defined: for total protein, from 6 to 8 per cent; albumin, from 4 to 5.5 per cent; globulin, from 1.4 to 3 per cent. Globulin in excess of 2.5 per cent was found only in the winter months and may represent effects of respiratory infections which are so prevalent in this climate at that season.

Total proteins have been determined 542 times in 374 patients suffering from various diseases which do not affect directly the cardiovascular or renal systems, and in 215 of these subjects 332 determinations of protein fraction have been made. The data have been analyzed from various points of view and the following conclusions have been drawn.

The relation between edema and the estimated protein osmotic pressure is in keeping with the theory of Starling.

Reductions of albumin can be connected almost invariably with malnutrition.

Increases of globulin are found with infections, especially suppurative processes and ulcerative tuberculosis or syphilis; in connection with certain tumors, particularly myelomata, and in cirrhosis of the liver.

The study has thrown no light on the sites of production of the two protein factors.

TRICHINIASIS, The Use of an Intradermal Test in the Diagnosis of, McCoy, O. R., Miller, J. J., Jr., and Friedlander, R. D. J. Immunol. 24: 1, 1933.

Of 36 persons ill with trichiniasis (i.e., two to six weeks after infection) 25 (70 per cent) gave positive immediate skin reactions following intradermal injection with 0.1 c.c. of a 1:10,000 dilution (in terms of dry weight of powder) of a saline extract of *Trichinella spiralis* larvae. An additional 8 reacted to a 1:500 dilution of the antigen making a total of 33, or 92 per cent positive.

Of 39 persons tested from three to twenty-two months after infection, 19 (49 per cent) reacted to the 1:10,000 dilution and an additional 12 were positive to a 1:500 dilution, a total of 31 or 80 per cent positive.

Of 13 persons ill from three and one-half to seven and one-half years prior to the test, 3 (23 per cent) were positive to the 1:10,000 dilution, and 5 more reacted to the 1:500 dilution, a total of 8 or 62 per cent positive.

One hundred and four control persons from Rochester, N. Y., showed an incidence of 9 per cent positive with the 1:10,000 dilution and a total of 18 per cent positive with both the 1:10,000 and 1:500 dilutions. Forty-seven control persons from San Francisco, Calif., showed an incidence of only 4 per cent positive to the 1:10,000 antigen and 6.5 per cent positive with both the 1:10,000 and 1:500 dilutions.

Ninety-two persons from southern Louisiana infected with *Trichuris trichiura* showed an incidence of 18 per cent positive with the 1:10,000 dilution and a total of 62 per cent with both the 1:10,000 and 1:500 dilutions.

From the data given in the present paper it appears fairly well established that about 90 per cent of persons ill with trichiniasis will give a positive skin test to the trichinella antigen provided the disease is sufficiently established. This period is apparently between two and three weeks after infection although in some cases a positive skin test may not appear until the fourth week.

The first consideration which complicates the interpretation of a positive trichinella skin test is the possibility that a previous light infection which has nothing to do with the present illness is responsible for the sensitization. This possibility is somewhat reduced by the fact that there seems to be a tendency to lose sensitivity over a period of years following infection.

Another consideration which must be thought of in the interpretation of a positive trichinella skin test is the possibility that the reaction is a Group I due to infection with *Trichuris trichiura*. The data show that most of these group reactions occurred with the 1:500 dilution of the antigen, but that a few probably occurred also with the 1:10,000 dilution. From the series of control tests performed, the authors are of the opinion that worm infections, other than trichuris, allergic disturbances, or other ordinary diseases are not an appreciable factor in causing group reactions to the 1:10,000 dilution of the trichinella antigen. It is possible, however, that in isolated instances group reactions may occur. In such cases it would probably be advisable to perform control tests with other proteins.

From a practical standpoint the trichinella skin test cannot be considered a certain diagnostic test. It appears that a negative test will probably prove more useful in ruling

out the diagnosis of trichiniasis than a positive one in establishing it. A positive test, however, definitely strengthens the evidence for the diagnosis and may justify further search for the parasite. Positive tests obtained with antigen dilutions of 1:10,000 or higher are of more definite diagnostic value than those obtained with lower dilutions. A year's experience in using the skin test in hospitals where trichiniasis not infrequently occurs leads the present authors to the conclusion that it may be a valuable aid in the diagnosis of the disease.

TRICHINOSIS, A New Jersey Outbreak of, With Report of a Case Complicated by Femoral Thrombosis, Kilduffe, R. A., Barbash, S., and Merendino, A. G. Am. J. M. Sc. **186**: 794, 1933.

A case of trichinosis complicated by femoral thrombosis is reported together with the particulars of an outbreak arising from the ingestion of homemade sausage and involving 43 persons.

TRICHINOSIS, Bachman Intradermal Test in Human, Kilduffe, R. A. Am. J. M. Sc. **186**: 802, 1933.

The results of the application of Bachman's skin test applied to 33 individuals exposed to trichinosis are reported and analyzed, from this analysis the author concluded that in the study of human trichinosis:

1. The demonstration of eosinophilia is not only technically simpler than the demonstration of the skin test, but always feasible, whereas the skin test, requiring an antigen difficult to prepare, is feasible only when the antigen is available.

2. In point of delicacy and constancy of appearance eosinophilia serves as a reliable index of trichinosis in the human being.

3. The Bachman skin test in the study of human trichinosis presents no practical advantages over the demonstration of eosinophilia.

NEUTROPENIA, Benign and Malignant, Beck, R. C. Arch. Int. Med. **52**: 239, 1933.

Because of its comprehensive survey of the subject this paper should be read in the original, as its character precludes a satisfactory abstract. The summary following indicates the completeness of this review, which is followed by a report of 4 cases.

The subject of benign and malignant neutropenia has been covered, and this information has been compiled in more or less textbook fashion.

Many names have been proposed for this disease entity, benign and malignant neutropenia seeming to be the most appropriate.

The two groups, benign neutropenia and malignant neutropenia, have been further subdivided into primary and secondary types, depending on the etiology. A classification is based on this method of distinction and on the clinical course, which tends to give a mental picture of the types that make up this disease.

A review of the physiology of granulopoiesis is given, since the accuracy in interpreting the blood picture in primary benign and malignant neutropenia is dependent on an understanding of the underlying mechanism of granulopoiesis.

A review of the theories of pathogenesis of benign and malignant neutropenia is covered, and a theory is formulated based on the underlying pathology and physiology of granulopoiesis. It is suggested that in each case of neutropenia it would be advisable to determine the etiology and underlying changes in the bone marrow. Biopsies on specimens of bone marrow in all cases before treatment is given would prove or disprove the theory of an endogenous disturbance of chemotactic and maturation factors for granulocytes as being the cause of this disease.

The pathology of the characteristic lesions has been covered, and complete laboratory data given.

Symptoms, diagnosis, differential diagnosis, and prognosis have been discussed.

A section is devoted to a theoretical consideration of the treatment for this disease, as well as a review of all forms of treatment to be found in the literature. The rationale of these treatments, when possible, has been discussed. The theory is advanced that a specific treatment for malignant neutropenia involves an investigation of the chemical maturation factors which determine the granulocytes.

SILICOSIS, The Pathology of So-Called Acute, Gardner, L. U. Am. J. Pub. Health 23: 1240, 1933.

A histologic study of 15 cases of so-called "acute" silicosis has been reported. This was based upon examination of the lung tissues of 9 tunnel workers exposed for from nine to thirteen months, 3 sand blasters, for twelve to seventeen months, and 3 sand pulverizers on whom satisfactory histories of exposure were not obtained. Chemical analysis demonstrated that their lungs contained as much silica as those of South African gold miners employed for long periods. Their alleged occupational exposures did not exceed seventeen months, a contention which is supported by the early age of the group as a whole. These men died of infection from nine to twenty-one months after ceasing work. In 11 of them the infection was considered to be definitely tuberculous, and in 2 others it was probably so. The other 2 had unresolved pneumonias. All but 1 of them, a tunnel man for thirteen months, presented microscopic lesions characteristic of silicosis, but in no case was the disease sufficiently far advanced to be diagnosed on gross examination of the fixed tissues. The character and distribution of the changes did not resemble that usually seen in silicosis among miners and others who have died after prolonged exposures. This silicosis was characterized: (1) by masses of small nodules embedded in broad sheets of fibrous tissue surrounding the pulmonary lymphatics instead of isolated conglomerate nodules along the trunks and in the pulmonary parenchyma; (2) by a generalized fibrous thickening of the alveolar walls, which is generally considered to be a late manifestation; and (3) by the absence or only slight involvement of the mediastinal lymph nodes which are usually replaced by fibrosis before extensive changes appear in the lung. These differences were interpreted as due to the inhalation of such excessive amounts of fine dust that little of it could be eliminated by the lymphatics. In most cases the tuberculous complication was so acute that it probably developed after the reaction to the dust was well established, and thus failed to influence the picture greatly.

The acuteness of the infection was explained by the fact that the majority of the men were young negroes with presumably little immunity to tuberculosis. Although there is histologic evidence of silicosis, atypical in character, it seems doubtful whether there is justification for describing the process as "acute." At least this should only be done after serial roentgenograms together with postmortem examinations have demonstrated the outcome of the allegedly heavy exposures to silica. The difficulty in properly evaluating the element of infection in the tissues suggests the need for caution in the interpretation of roentgenograms.

SILICOSIS, Silica Content of Lungs of a Group of Tunnel Workers, Smith, C. S., and Wikoff, H. Am. J. Pub. Health 23: 1250, 1933.

The lungs of 9 workmen engaged in the same tunnel project have been analyzed.

There is a striking correlation between the per cent SiO_2 in the dried lung substance and the severity of silicosis as determined histologically.

The per cent SiO_2 in the dried lung substance is a better criterion of the intensity of silicosis than is the per cent of silica in the total ash.

BLOOD, Is Halometry Reliable? Chaudhuri, H. P. Indian J. M. Research 21: 315, 1933.

From an extensive statistical and comparative study, it is concluded that the halometric determination of the size of erythrocytes furnishes reliable data.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

An Outline of Immunity*

AS THE author pertinently remarks in his Preface, there is probably no subject in a less satisfactory position than that labeled "Immunity." "A hybrid creature, not quite sure of its true affiliations; wandering uneasily between the Departments of Pathology, Bacteriology, and Hygiene; at times attempting to set up a house all by itself; a vigorous infant among the sciences, just rising fifty years, like most young and vigorous things, it is a little uncertain in its movements, a little confused in its mind, and at times more than a little incoherent in its speech."

Few who are familiar with the many, and at times not a little startling developments in the field of immunity within comparatively recent times, will deny the aptness of Professor Topley's characterization of his subject. That he has been able to present as clear and as comprehensive a survey of so complicated a subject as he has given in the present volume is a matter upon which the medical profession may well congratulate him and itself.

This is a book which should prove of inestimable value not only to the student and laboratory worker, but to the physician at large who, obliged almost daily to utilize the phenomena of immunity in the treatment and prophylaxis of disease, is obliged also to do so often without clear apprehension of the principles involved and their practical application.

In the twenty-one chapters of this volume the present concepts of immunity are reviewed, summarized, and correlated in a comprehensive and authoritative way. The book, far from being abstruse and concerned with hypothesis, is eminently practical in its outlook and consistently concerned with the practical aspects of immunity as related to the prophylaxis and treatment of disease.

An excellent feature is the succinct yet clear summary at the end of each chapter which, together with the excellent index, renders the contents of the volume readily accessible.

This book may be highly recommended as serving a useful purpose and as presenting a highly practical survey of an important and complicated subject.

Coccidia and Coccidiosis†

COCCIDIA, in their relation to disease in animals and man, have attracted attention in many and diverse fields as witnessed by the numerous papers concerning coccidiosis which have appeared in journals of various types.

This field, however, is a highly specialized one, the literature of which is so widely scattered as to be almost inaccessible to even the specialist with good library facilities.

This monograph, therefore, serves a most useful purpose in bringing together under one cover descriptions and illustrations of the different coccidia found in various hosts and presents, in addition, the results of investigations in the study of "host-parasite-relationships."

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*An Outline of Immunity. By W. W. C. Topley, Professor of Bacteriology and Immunology, University of London. Cloth, pp. 415, 37 figures, 63 tables. William Wood & Co., Baltimore, Md.

†Coccidia and Coccidiosis of Domesticated, Gram, and Laboratory Animals, and of Man. By Elery R. Becker, D.Sc., Associate Professor of Protozoology, Iowa State College. Cloth, pp. 147, 25 figures. Collegiate Press, Ames, Iowa.

Throughout the book much attention has been paid to discussions of those phases of coccidiosis which have been the subject of misconception in the past, such as host-specificity, the nature of species, the relationship to disease, immunity, methods of reducing infection, and the tolerance of the oocysts.

There are numerous illustrations and an appendix on technic together with an extensive bibliographic index and a general index render the contents of the book readily accessible.

To the biologist, the pathologist, the practitioner, and the breeder, this book should be of interest and value as correlating in a systematic manner the present information available concerning a subject about which there has been much confusion in the past.

Recent Advances in Vaccine and Serum Therapy*

THE purpose of this book is to survey the present state of knowledge regarding the employment of vaccines and sera in the prevention and treatment of disease.

Few will deny that there has been and still is more or less clinical confusion in this field and the present volume is, therefore, not only timely but fills, as well, a place long empty in the physician's reference library.

The book is divided into two parts. Part I, written by Dr. Petrie, is concerned with the prevention and treatment of disease by means of sera and with the specific prophylaxis and treatment of virus diseases in man and animals.

Part II, written by Professor Fleming, surveys the prevention and treatment of disease by means of vaccines.

An extraordinary amount of work has been done in both these fields, and the physician, laboratory worker, and student here finds it admirably surveyed and correlated.

The authors have not been satisfied merely to list or abstract the numerous investigations which have influenced present concepts in these fields, but present them in the light of their own experience, as a coherent and authoritative story, well conceived and well written.

The list of references presented at the end of each chapter evidences a careful and painstaking review of the literature and the extensive subject index as well as a complete and separate author index render the contents of the book readily accessible.

This volume should be on the reference shelf of every worker interested in this field and may be classed as indispensable for the physician who desires to utilize either sera or vaccines in an intelligent manner.

It may confidently be accepted as a valuable as well as highly practical reference work in this important field and recommended without reserve to the practicing physician.

Manual of Determinative Bacteriology†

THE *Manual of Determinative Bacteriology* sponsored by the Society of American Bacteriologists and edited by Dr. Bergey has long been indispensable to clinical and bacteriologic laboratories and requires neither introduction nor labored comment.

This new fourth edition is enlarged by nearly 100 pages by amplification in the description of species and by the addition of about 50 species and one new genus.

Despite the thorough revision and the increased size of the book the price is unchanged. Were it twice as much the book would still be invaluable.

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*Recent Advances in Vaccine and Serum Therapy. By Alexander Fleming, Professor of Bacteriology, University of London, and G. F. Petrie, Bacteriologist-in-Charge, Serum Department, Pasteur Institute, Elstree. Cloth, pp. 463, 5 illustrations. P. Blakiston's Son and Co., Philadelphia, Pa.

†Manual of Determinative Bacteriology. By David H. Bergey. Cloth, pp. 664. Fourth edition. Williams & Wilkins Co., Baltimore, Md.

Practical Methods in Biochemistry*

WHILE intended primarily for medical students this manual should also prove useful to the physician, not only as a technical manual, but also as a work of reference concerning the principles and methods of biochemistry which now occupy so prominent a place in the practice of medicine.

The style is clear, simple, and easily understandable and the volume may be recommended as a comprehensive and reliable guide.

A Textbook of Bacteriology†

FEW textbooks of bacteriology have attained a more lasting or more deserved popularity than that of Hiss and Zinsser, the new seventh edition of which, prepared by Zinsser and Bayne-Jones and dedicated to the memory of Dr. Hiss, now makes its appearance.

So familiar is this excellent text as a comprehensive and authoritative presentation of this subject that little need be said by way of introduction.

While extensively revised and in many ways rewritten, the original purpose of the book has been maintained, to present not only a treatise on the fundamental laws and technic of bacteriology, as illustrated by their application to the study of pathogenic bacteria, but also to serve as a manual of infectious diseases.

The scope of the work is indicated by the titles of the nine main sections into which it is divided: Part I, The General Biology of Bacteria and the General Methods of Bacteriology; II, Infection and Immunity; III, Pathogenic Microorganisms; IV, The Rikettsia Diseases; V, The Spirochetes; VI, Medical Mycology: Molds, Yeasts, Actinomycetes, and Pathogenic Fungi; VII, Diseases Caused by Ultramicroscopic Viruses, the Exanthemata, and Diseases of Uncertain Etiology; VIII, Outline of Diagnostic Methods of Medical Protozoology; IX, Technical Methods of Bacteriology, Immunology, and Serology.

The revision has been thorough and extensive and embodies consideration of all the many and marked advances which have been made within the last ten years in the field of bacteriology and immunology and includes a discussion of the contributions made in 1933 and the early part of 1934.

As before, the book constitutes an outstanding contribution in this field and one without which no reference library can be complete.

It can be enthusiastically recommended without reserve and, without doubt, will receive the same general approval as its predecessors.

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*Practical Methods in Bacteriology. By F. C. Koch, Professor of Physiological Chemistry, University of Chicago. Cloth, pp. 282. 17 figures. William Wood & Co., Baltimore, Md.

†A Textbook of Bacteriology. By Hanz Zinsser, M. D., Professor of Bacteriology and Immunology, Harvard University; and Stanhope Bayne-Jones, M.D., Professor of Bacteriology, Yale University Medical School. Ed. 7, cloth, pp. 1226, 174 illustrations. D. Appleton-Century Co., New York.

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EDITORIALS

Barnacles

HUMAN psychology perhaps to some extent may be reflected in its proverbs so that the time-worn sayings concerning the silver lining and the ill wind that, despite itself, blows some one some good all may justify the dictum that "hope springs eternal in the human breast."

Certain it is that not infrequently in the history of civilization great catastrophes, extraordinary plagues and similar events, regarded at the time as crushing and devastating blows to fortune, have been instead the starting points for tremendous advances and discoveries and have initiated a cycle of events productive of far-reaching benefits to humanity.

If history, as it is reputed to do, again repeats itself, perhaps the depression into which the world at large has been plunged for some years past and from which it shows faint but indubitable signs of beginning to emerge, may in its turn be productive of direct and indirect benefits.

It was inevitable under the circumstances that the social world should, in the face of its apparent collapse, take stock of the mechanism which holds it together and, as it were, makes it go; and very naturally medicine, in which term is included all that has to do with the management and control of disease and its congeners, has not escaped a critical glance.

Community responsibility for the care of the sick is an essential part of the development of civilization, for the discharge of which a huge structure has developed, ranking in importance, not only because of its essential necessity but also because of its economic magnitude and ramifications, with any of all those of which modern civilization can boast.

Hospitals are an integral part of this structure and, although not commonly regarded as "Big Business," properly belong in this category; for the seven thousand hospitals in the United States in round figures represent a capital investment of more than three billions of dollars, a capital sum exceeded by but few of the great industries.

It is entirely probable that there is little general appreciation of the fact that the average one hundred bed hospital represents an average capital investment of one million dollars and that the average investment per bed in accordance with the size and type of hospital, for acute or chronic diseases or including research facilities, varies from $2,500 to more than $10,000.

On the basis of the investment involved hospitals must be regarded as a business, "Big Business," the business of medical care, and the fact that this business ship within recent years has been laboring rather heavily in a heavy, stormy economic sea, as it were, has focussed attention upon this structure and the proposal by many individuals and groups of various measures to allay the storm and set the vessel once more on an even keel.

Oceans of ink have already been spilled in the discussion of what has popularly become familiar as "the cost of medical care" and the end of the avalanche of words and windy dispute is far from near, nor will it be found herein, for the subject is not to be dismissed within the limits of an editorial. Nor is any discussion here intended of the relation of hospital costs to the general costs of illness, important as it is. Those are matters deserving abler pens; these are but casual observations.

Above, the hospital structure with a striking lack of originality has been likened to a ship and a meager acquaintance with matters nautical—gathered, it must be confessed, from perusal of such authorities as Marryatt, the varied tales of Conrad, *Moby Dick*, and the reading (repeatedly) of *Treasure Island*—allow us to state with some confidence that when the good ship no longer shows her accustomed speed but, instead, merely lumbers along, wallowing ungracefully, the scraping from the bottom of barnacles and accumulated débris was a very common custom apparently productive of the desired result.

Can it be that hospitals in the course of time have accumulated any barnacles? Can it be that this huge structure has drawn to itself by custom or habit any accretions not perhaps essential per se to its efficiency or existence?

The business of hospitals, as already said, is the business of medical care and hence indissolubly associated with the practice of medicine and the physi-

cian. And physicians of late have had it brought rather forcibly to their attention that to some extent, varying under different circumstances and in different localities, the practice of medicine seems to have passed or to be passing from the hands of the physician into the hands of the hospital.

Now, while it has been possible in the past, and is still possible in the present, to carry on the business of taking care of the sick without the hospital, it is perhaps sometimes minimized if not overlooked that the hospital without the physician is rather a helpless if not useless institution. It is conceivable that there are hospital boards and perhaps, even superintendents to whom such an idea would appear preposterous, and it is not strange that this should be so when one pauses to consider how little, relatively speaking, the physician has to do with the running of hospitals.

True, as a dispensary worker he gives untold hours of his time freely and without remuneration to the care of patients, not a few of whom are without moral right or justification for such free treatment, and after often a lengthy period of service in this capacity, he graduates to the dispensing of free care under like circumstances in the wards, and sometimes, as at present, there comes some vague wonderment as to just how all this came about and whether or not he may not thereby be committing a sort of slow economic hara-kiri.

If, as is not at all beyond the bounds of possibility, the practice of medicine is to become a function of the social organization either permanently or temporarily until such time as the futility of such a program, despite its theoretical advantages, becomes evident in practice, the physician may well wonder just what place will be his in relation to the hospital.

Although the business of the hospital is the business of taking care of the sick, the physician is seldom indeed found directing its destiny in that respect, for custom seems to have decreed that hospital superintendents are most often graduate nurses. Just why this should be so; what basis there is for assuming that the training of a nurse implies executive ability or the ability to supervise rather than to assist in the care of the sick, is, it must be confessed, somewhat difficult to ascertain.

Yet the fact remains that perhaps a majority of hospitals of average size have nurses as superintendents, not infrequently directing the Board of Managers as well as the hospital and casting a supervisory eye over the activities of the staff. And, as some cynic once remarked: Hospitals are sometimes mismanaged because they are Miss managed.

If, in his extremity, the physician turns to the field of anesthesia, certainly an important one, properly a specialty of medicine and so largely regarded in England, here, too, he finds the nurse rather firmly ensconced.

There is no place for him on the Board of Managers, for it is only very recently that Staff representation has been granted as a favor.

He may strongly suspect that this or that free patient is well able to pay for treatment and belongs in the doctor's office rather than the dispensary, but the nurse or the lay social service worker has decreed otherwise, and that's that!

He finds himself arraigned and accused of grave responsibility for the high costs of medical care, and finds it somewhat difficult to reconcile the accusation with the fact that the money earmarked for payment of his bill has been swallowed up in payment of the hospital's.

And as the economic storm rages about him, he finds himself working for the hospital, carrying on the practice of medicine for it and nourishing thereby a host of lay personnel. Neither skipper, mate, nor even bos'n, small wonder that he wonders whether it were not better to be merely another barnacle.

—*R. A. K.*

The Anemia of Pregnancy

WHILE the fact that pregnancy may be accompanied by a varying degree of secondary anemia is more or less commonly recognized, it is only within recent times that, possibly as an aftermath of studies in prenatal care, much attention has been paid to its presence or much effort expended upon the determination of its degree.

We refer, not to the severe hemolytic anemia, sometimes spoken of as the pernicious anemia of pregnancy, but to the definite secondary, though not always marked anemia which seems to be the result of pregnancy per se.

The pernicious or hemolytic anemia of pregnancy is encountered during the later months of pregnancy and the puerperium. Insidious in onset, it may reach a marked degree without the production of marked symptoms and thus may escape detection until attention is drawn to it by symptoms closely resembling those seen in true pernicious anemia.

The hemolytic anemia of pregnancy is the result of the action of a syncytial hemolysin arising in the ectodermal cells of the chorion. Under normal conditions as pregnancy progresses an antihemolysin is formed in the maternal blood but when, for any reason, its formation is inhibited, the hemolytic anemia of pregnancy is the result.

Fortunately, in view of its rather high mortality, this form of anemia in pregnancy is rather uncommon.

The less marked but still definite secondary anemia of pregnancy, on the other hand, is very common and of definite significance although often overlooked when cytologic examinations of the blood are neglected as a part of the prenatal survey.

The findings of practically all observers who have reported upon this subject present a remarkable conformity.

Bland and Goldstein,[1] in a study of 50 cases, found a hemoglobin content ranging from 42 to 78 per cent (Dare) and erythrocyte counts of from 2.36 to 3.98 million cells per cubic millimeter.

In a second study of 300 cases, 100 being private cases, Bland, Goldstein and First,[2] found red cell counts below 3.5 million in 33.3 per cent of cases during the first two trimesters of pregnancy, 55.7 per cent in the third trimester, and 26 per cent during labor.

Galloway[3] in a study of 382 cases found average hemoglobin values (Sahli) of 73, 69, and 66 per cent for the first, second, and third trimesters respectively, and erythrocyte counts of 4.05, 3.94, and 3.87 millions per cubic millimeter evidencing both a chlorotic type of anemia as well as a definite progression as the pregnancy advanced.

Practically the same findings are reported by Kilduffe[4] in 300 cases as shown in Table I.

TABLE I

DURATION OF PREGNANCY, MONTHS	NO. CASES	AVERAGE HB. (HADEN) GM.	PER CENT	AVERAGE R.B.C. MILLIONS PER C. MM.
1 – 3	12	10.07	73	4.50
4 – 6	72	9.93	72	3.73
7 – 9	216	9.10	66	3.73

It is unnecessary to cite the many other studies available, as all present comparable findings so that it may be accepted as proved that an anemia secondary to pregnancy per se is present in from 50 to 60 per cent of all cases, which shows a tendency to be somewhat chlorotic in type and a definite tendency to progress pari passu with the progression of the pregnancy.

The mechanism responsible for its production has been the subject of much discussion, many different theories having been advanced, none of which, however, have been definitely proved.

Thus, it has been attributed to hydremia producing a decreased iron content of the corpuscles, which would account for its chlorotic type; to poor hygiene or the presence of such diseases as nephritis, syphilis, and tuberculosis; to the continuation of a preexisting anemia; to focal infections; and to the expression of an "individual disposition" in accordance with the general physical well-being of the individual patient.

As stated, however, none of these conceptions has been susceptible of clear-cut, definite or indisputable proof.

The possible relation of this secondary anemia of pregnancy to the presence of the syncytial hemolysin held to be responsible for the hemolytic anemia mentioned above but inhibited in the case of the secondary by the later production of an antihemolysin is one of the oldest theories but, like all the others, still remains but a theory while its occurrence in the higher classes of patients, usually seen as private cases, robs environment factors of all significance.

Many investigators of this question assert that the anemia spontaneously disappears after delivery but many others are unable to find conclusive evidence of the correctness of this contention.

The matter still remains a problem awaiting solution, the present status of which may be thus summarized:

1. Pregnancy, per se, produces a secondary anemia somewhat chlorotic in type which progresses pari passu with the progression of pregnancy.

2. No satisfactory or demonstrable etiologic mechanism has yet been found.

3. In view of these facts, cytologic blood studies should be a part of routine prenatal care.

4. All patients with a hemoglobin below 75 per cent and erythrocyte counts under 3.5 million should be particularly observed and therapeutic measures instituted immediately upon the discovery of the deficiency. It is advisable also that such patients be watched for several months after delivery for evidence of bone marrow changes.

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—*R. A. K.*

CORRESPONDENCE

Ancther Simple Rctary Incculating Table

To the Editor:

Whether one has many or few Petri plates to stroke, a rotary table gives very satisfactory results and is a great convenience.

Quite a number of designs of such a piece of apparatus have appeared in the recent literature, requiring for construction the services of anyone from a blacksmith to a Swiss watchmaker.

We have found that the simplest and most satisfactory method of constructing such a machine is to take a two-dollar bill and go out and buy an old discarded portable Victrola. (Since the advent of Radio, one can sometimes get two dollars for taking the machine away.) If the reader cannot improvise from several old records ("She Knows Her Onions" or "On the Beach at Waikiki" preferred) and some thumb tacks, a means of holding the Petri plate on the revolving turntable, he should not be trusted with a platinum loop. With the brake the speed may be adjusted to suit the bacteriologist's artistic temperament.

We have used our machine for four or five years with utter satisfaction.

Eric A. Fennel, M.D.

Honolulu, Hawaii, April 10, 1934.

INDEX TO VOLUME 19

AUTHORS INDEX

In this index following the author's name the title of the subject is given as it appeared in the JOURNAL. Editorials are also included in the list and are indicated by (*E*).

A

ABBOUD, M. A. (*See* Gohar and Abboud), 751
ABERNETHY, R. J., RUSSELL, E. R., AND THIENES, C. H. The estimation of ethyl alcohol in brain, 1014
ABRAMOWITZ, E. WILLIAM. (*See* Williams, Abramowitz, and Killian), 1213
ABRAMSON, DAVID I., CRAWFORD, J. HAMILTON, AND ROBERTS, GEORGE H. The relationship between the coronary blood supply to experimentally produced ventricular lesions and the resulting electrocardiographic alterations, 1040
AMES, AZEL. (*See* Herrmann, Ames, and Tapke), 411
ANDERSON, ARTHUR K. (*See* Pritham and Anderson), 892
APPLETON, J. L. T., JR. (*See* Peterson and Appleton, Jr.), 1079

B

BAILEY, JOHN F. (*See* Poe and Bailey), 40
BAKER, CHARLES P. (*See* Myers, MacQuiddy and Baker), 462, 468
BAKER, HENRY. (*See* Healy and Baker), 133
BEATTIE, MARGARET. A new method for the production of antisheep hemolysin, 666
BEAVER, DONALD C., AND JOHNSON, WALTER R. A probable unusual case of Hodgkin's disease, 625
BENJAMIN, LIONEL. The purification of gum dammar for use as a mounting media, 325
BENSLEY, E. H. Estimation of plasma bilirubin, 1122
BERCOVITZ, Z. Modified technic for making Wright's blood stain, 438
BEREGOFF, PAULINE. The Kolmer, Kahn, and Meinicke reactions in the tropics, 780
—. The Schilling differential blood count in tropical diseases, 644
BERNHARD, A. A simple and efficient reflux condenser, 314
BIRCHWOOD, E. Benign adenoma of the sweat glands, 586
BITTMAN, FLORENCE R. (*See* Nammack and Bittman), 1094
BLAIR, ALLAN W., AND CARMICHAEL, EMMETT B. Cage for mice and rats, 1020
BLISS, A. RICHARD, JR., AND MORRISON, R. W. A comparative study of certain xanthine diuretics, 248
BOERNER, FRED, AND LUKENS, MARGUERITE. A modification of the Lange colloidal gold test, 1007
BOOTH, J. D. (*See* Hemingway, van de Erve, and Booth), 738
BORSOOK, HENRY. (*See* Keighley and Borsook), 650
BOWLES, J. A. C. (*See* Partridge, Bowles, and Goldfeder), 1100
BRAMS, W. A., AND GOLDEN, J. S. Observations on the vascular response to drainage of ascites, 948
BRICE, ARTHUR T., JR. The tryptophane content of blood serum, 1113
BRIGGS, A. P. (*See* Broun and Briggs), 886
BROOKS, HARLOW. Medicine of the American Indian, 1
BROUN, G. O., AND BRIGGS, A. P. Hemoglobin and blood cell relations as determined by iron and oxygen capacity methods, 886
BROWN, CHARLES D. A simple and efficient electrical tool for sealing museum jars, 197
BROWN, HERMAN. (*See* Davis and Brown), 1049
BROWN, MARSHALL S., JR., AND RALLI, ELAINE P. The dietary treatment of undernutrition, 1169
BRUGER, MAURICE, MIRSKY, I. ARTHUR, AND MEMBER, SAMUEL. The variations of the urea, total nonprotein nitrogen and chloride concentration in the blood following glucose ingestion, 474
BULLOWA, JESSE G. M., AND WILCOX, CLARE. Pneumococcus chain formation, 1156
BUNKER, JOHN W. M. (*See* Harris and Bunker), 390
BURGER, GEORGE N. The treatment of irritable colon with sodium ricinoleate, 234

C

CALDERONE, FRANK A. (*See* Friedman and Calderone), 1332
CAMPBELL, LEO K. Experiments in use of a mineral water in management of diabetes mellitus, 1088
—. The use of synthalin in diabetes mellitus, 1067
CARMICHAEL, EMMETT B. (*See* Blair and Carmichael), 1020
Chalek, I. (*See* Moen, Medes, and Chalek), 571
CHALLIS, FRANK E. (*See* Williams and Challis), 166
CHENEY, R. H. A simplified psychodometer, 1238
CHILLINGWORTH, FELIX P., HEALY, JAMES C., AND HASKINS, FRANKE E. Reflex eosinophilia, 486
COHN, MAURICE L. (*See* Corper and Cohn), 899
—. (*See* Corper, Cohn, and Hoper), 1179
COLE, VERSA V. (*See* Davis, Curtis, and Cole), 818
COLE, W. H., AND WOMACK, N. A. A simple and inexpensive apparatus for the determination of metabolic rate in guinea pigs, 540
COLEMAN, MARION B. (*See* Gilbert, Coleman, and Laviano), 225
COLMES, ABRAHAM. The perennial treatment of hay fever, 481
COOKE, W. E. The macropolycyte, 453
COOLEY, THOMAS B. (*See* Peterman and Cooley), 723, 743
CORPER, H. J., AND COHN, MAURICE L. Maintaining water and air balance during prolonged incubation, 899
—, —, AND HOPER, VYOLA J. Sodium taurocholate and the virulence of human tubercle bacilli, 1179
CO TUI, FRANK WANG. The combined effects of bile salts and oleic acid on choleresis, 567
CRAWFORD, J. HAMILTON. (*See* Abramson, Crawford, and Roberts), 1040
CRIMM, PAUL D., AND STRAYER, J. W. Vitamin therapy in pulmonary tuberculosis, 966
CROCKER, WALTER J. (*See* Rathmell and Crocker), 1206
CURTIS, GEORGE M. (*See* Davis, Curtis, and Cole), 818
—. (*See* Phillips and Curtis), 896
CUTTING REGINALD A. Dog-operating table, 1117

D

DACEY, H. GLADYS, AND KOROVIN, NATHAN. Intracutaneous reactions induced in guinea pigs inoculated with B. abortus, 589

DAVIS, CHESTER B., CURTIS, GEORGE M., AND COLE, VERSA V. Blood iodine studies, 818

DAVIS, GEORGE E., AND SHEARD, CHARLES. The spectrophotometric investigation and determination of bilirubin, 593

DAVIS, GRACE I., AND DAVIS, THOMAS W. The preservation of biologic specimens, 774

DAVIS, REUBEN, AND BROWN, HERMAN. Spinal fluid sugar determinations in experimental hypoglycemia of dogs, 1049

DAVIS, THOMAS W. (*See* Davis, Grace I., and Davis), 774

DECOURSEY, ELBERT. Coronary occlusion and sudden death, 1272

DEHN, WILLIAM M. (*See* Gibb and Dehn), 1018

DE TAKATS, G. (*See* Zimmerman, Gault, Halpern, and de Takats), 243

DOWDEN, C. W., MCNEILL, CLYDE, AND MCNEILL, J. D. A clinical study of blood iron and hemoglobin, 362

DUNN, F. LOWELL. The calibration of white blood cell dilution pipettes, 95

E

EAGLE, HARRY. Studies in serology of syphilis, 621

EISENBERG, A. A., AND WALLERSTEIN, HARRY. Chronic myelosis in children, 713

—, —, AND PLATO, AARON. Cytologic study of body fluids, 315

ELLENBERG, SAMUEL L. The sedimentation reaction in the newborn, 944

EMERSON, CHARLES P., JR., AND HELMER, O. M. A study of the salivary amylase in patients with pernicious anemia, 504

ENTWISLE, R. M. (*See* McClellan and Entwisle), 507

EPSTEIN, PAULINE. Special rack for Smith fermentation tubes and "U" tubes, 553

ESELIUS, ERIC. (*See* Sackett and Eselius), 546

EVELETH, DONALD F. Note on the determination of blood urea by direct nesslerization of a sodium tungstate—sulphosalicylic acid filtrate, 783

F

FALK, K. GEORGE. (*See* Klein, Greenwald, and Falk), 1324

FEEMSTER, ROY F. Simplifying the mechanics of bacterial filtration, 786

FERGUSON, JOHN A., MURDOCK, THOMAS P., AND WELCH, HENRY. The significance of streptococci that resemble diphtheroids recovered from blood cultures in subacute bacterial endocarditis, 1304

FISHER, L. W. Studies on hemolytic streptococci isolated from hemorrhagic smallpox, 280

FORBES, T. W. An improved electrode for the measurement of potentials on the human body, 1234

FRANKE, KURT W., AND FRANKE, W. ROYDEN. A metabolism cage for rats, 669

FRANKE, W. ROYDEN. (*See* Franke, Kurt W., and Franke), 669

FRIEDMAN, MAX M., AND CALDERONE, FRANK A. Quantitative determination of chloral hydrate in blood and urine, 1332

FRIEDMAN, RAYMOND. (*See* Landt and Friedman), 1091

FRITZELL, AGNES I. (*See* Goldhamer and Fritzell), 172

G

GATES, LOUIS W. (*See* Klarmann, Shternov, and Gates), 835

GAULT, J. T. (*See* Zimmerman, Gault, Halpern, and de Takats), 243

GIBB, W. E., AND DEHN, WILLIAM M. The Diazo reaction for detection of certain local anesthetics in urine and in tissues, 1018

GILBERT, RUTH, COLEMAN, MARION B., AND LAVIANO, ALICE B. A study of the granular and floccular types of agglutination with B. typhosus, 225

GLASSBERG, B. Y. Gauging the dose of insulin, 1173

GLICK, DAVID. Note on the procedure in the Kuttner-Lichtenstein microcolorimetric method for the determination of organic phosphorus, 1012

GOERNER, ALFRED, AND HALEY, FRANK L. Demonstration of penetration of an antiseptic dye into the tissues of genitourinary tract, 735

GOHAR, M. A., AND ABBOUD, M. A. The causative organisms of pneumonia in children in Egypt, 751

GOLD, JOHN. (*See* Kasman, and Gold), 361

GOLDEN, J. S. (*See* Brams and Golden), 948

GOLDFEDER, ANNA. (*See* Partridge, Bowles, and Goldfeder), 1100

GOLDHAMER, S. MILTON, AND FRITZELL, AGNES I. A study of the red blood cell count and hemoglobin in the adolescent male, 172

GOTTLIEB, R. Blood studies in hyperthyroidism, 371

GOWER, W. E., AND VAN DE ERVE, J. An automatic and graphic method of recording diuresis, 81

GRANET, EMIL. Studies of the mechanism of pain in peptic ulcer, 47

GREAVES, AUBREY V. A new microscope adapter for the hand spectroscope, 755

GREENE, ROBERT A., AND ROEHM, RICHARD R. A combination condensing and receiving vessel, 1116

GREENWALD, CHARLES K. (*See* Klein, Greenwald, and Falk), 1324

GRUNER, O. C. Intracellular structures in monocytes in cases of malignant disease, 917

GRUSKIN, BENJAMIN J. (*See* Rawls, Gruskin, Ressa, and Jordon), 830

GUEST, GEORGE M., AND SILER, VINTON E. A centrifuge method for the determination of the volume of cells in blood, 757

H

HADEN, RUSSELL L. The determination of hemoglobin by the iron content method, 406

HANKEY, ROY A. An improvised bacteriologic incubator, 1098

HALBACH, ROBERT MCCULLEY. The diagnosis of surgical tuberculosis, 309

HALEY, FRANK L. (*See* Goerner and Haley), 735

HALPERN, S. S. (*See* Zimmerman, Gault, Halpern, and de Takats), 243

HARGROVE, M. D., AND MATHEWS, W. R. A fatal case of sickle-cell anemia with autopsy findings, 126

HARRIS, ROBERT S., AND BUNKER, JOHN W. M. Variability in the corn component of a rachitogenic diet, 390

HARWOOD, ROBERT UNWIN. A method for the separation of the principal constituents of bile, 1003

HASKINS, FRANK E. (*See* Chillingworth, Healy, and Haskins), 486

HAYNES, FLORENCE W. The determination of serum protein by measurement of volume of precipitate, 1320

HEALY, JAMES C. (*See* Chillingworth, Healy, and Haskins), 486

—, AND BAKER, HENRY. Blood catalase in heart disease, 133

HELLEBRANDT, FRANCES A. (*See* Kelso and Hellebrandt), 1105

HELLER, V. G., AND PAUL, HENRY. Changes in cell volume produced by varying concentrations of different anticoagulants, 777

HELMER, O. M. (*See* Emerson, Jr., and Helmer), 504

HEMINGWAY, M. W., VAN DE ERVE, J., AND BOOTH, J. D. Some experimental studies on nembutal (pento-barbital-sodium), 738
HERRMANN, LOUIS G., AMES, AZEL, AND TAPKE, ROBERT J. Observations upon the Lipokrit method for the determination of the lipoid content of blood, 411
HICK, FORD K., AND MORTIMER, HAROLD M. Carcinoma of pancreas, 1058
HILL, FREDERICK C., NEIGUS, IRWIN, AND WILHELMJ, CHARLES M. A method of preparing duodenal pouches for use in experimental work, 1126
HINES, LAURENCE E., AND MAHER, CHAUNCEY C. A study of quinidin effects on ambulatory patients with auricular fibrillation, 232
HINTON, WILLIAM A. Choosing a serum test for syphilis, 275
HIRSCHBERG, NELL. An evaluation of three methods for the demonstration of tubercle bacilli for use in hospital routine, 429
HOFFMAN, WILLIAM S., AND JACOBS, H. R. D. The partition of potassium between the serum and corpuscles in health and disease, 633
HOLCK, HARALD G. O., KANAN, MUNIR A., AND HOMMA, HANS. Intravenous lethal doses of amytal in the dog and rabbit and a table of animal dosages compiled from the literature, 1191
HOMMA, HANS. (*See* Holck, Kanan, and Homma), 1191
HOPER, VYOLA J. (*See* Corper, Cohn, and Hoper), 1179
HOWES, EDWARD L. (*See* Jern, Howes, and Meleney), 1257
HOYT, ANSON. Studies on rubber glove sterilization and the use of sterility indicators, 382
HUEPER, W. C. Histochemical studies of organs of tumor-bearing rats by the microincineration method, 1293
HUTCHESON, J. M. A simple autopsy stand for mice, 210

J

JACOBS, HENRY R. A practical method for the continuous administration of fluid intravenously, 768
JACOBS, H. R. D. (*See* Hoffman and Jacobs), 633
JERGESEN, FLOYD H., AND SIMONDS, J. P. The blood lipase in patients with peptic ulcer, 1054
JERN, HELEN ZAYTZEFF, HOWES, EDWARD L., AND MELENEY, FRANK L. Studies in Bacteriophage, 1257
JOHNS, F. M. A study of punctate stippling as found in lead poisoning of wild ducks, 514
JOHNSON, HARRY J., AND SHORT, JAMES J. Effect of smoking on skin temperature, 962
JOHNSON, WALTER R. (*See* Beaver and Johnson), 625
JONES, R. FRANK. (*See* Poindexter and Jones), 864
JORDON, MARIE. (*See* Rawls, Gruskin, Ressa, and Jordon), 830

K

KAMLET, JONAS. A simplified microdetermination of cholesterol in whole blood, serum, and plasma, 883
KANAN, MUNIR A. (*See* Holck, Kanan, and Homma), 1191
KASMAN, LOUIS P., AND GOLD, JOHN. Metaplastic changes in the prostate gland, 301
KATZ, LOUIS N. Observations on oxygen therapy, 1164
KEHOE, ROBERT A., AND THAMANN, FREDERICK. The behavior of lead in the animal organism, 178
KEIGHLEY, GEOFFREY, AND BORSOOK, HENRY. A rapid colorimetric method for multiple determinations of uric acid, 650
KEIL, H. L., AND NELSON, VICTOR E. The effect of various colloidal and crystalloidal metallic compounds in nutritional anemia of rat, 1083
KELLER, ALEXANDER G., JR. Determination of the stroke volume of the heart, 994
KELSO, L. E. A., AND HELLEBRANDT, FRANCES A. The recording electrodynamic brake bicycle ergometer, 1105
KENNEDY, PATRICK J. (*See* Moon and Kennedy), 295
KILDUFFE, ROBERT A. The anemia of pregnancy, 1358 (*E*)
—. Barnacles, 1355 (*E*)
—. Developments in the study of agglutinins, 1144 (*E*)
—. Have hospitals attained a standard of mediocrity, 1251 (*E*)
—. The laboratory diagnosis of undulant fever, 1029 (*E*)
KILLIAN, JOHN A. (*See* Williams, Abramowitz, and Killian), 1213
KING, ARTHUR G. The Friedman rabbit ovulation test in differential obstetric diagnosis, 1033
KINGERY, LYLE B. (*See* Woodward, Kingery, and Williams), 1216
KISÉ, YOSHIAKI AND OCHI, TAKUMI. Basal metabolism of old people, 1073
KLARMANN, EMIL, SHTERNOV, VLADIMIR A., AND GATES, LOUIS W. The bactericidal and fungicidal action of homologous halogen phenol derivatives and its "quasi-specific" character, 835
KLEIN, A. J., GREENWALD, CHARLES K., AND FALK, K. GEORGE. The refining of antiserums, 1324
KOHL, C. (*See* Shevky, Kohl, and Marshall), 689
KOLMER, JOHN A., RULE, ANNA M., AND MADDEN, BERNARD. Chemotherapeutic studies with sodium ricinoleate (soricin), 972
KOROVIN, NATHAN. (*See* Dacey and Korovin), 589
KRACKE, ROY R., AND PARKER, FRANCIS P. The etiology of granulopenia (agranulocytosis), 799
KRAUSS, WILLIAM. (*See* Nash and Krauss), 94
KRETSCHMER, OTTO S. The gram property of the acid-fast form of the tubercle bacillus, 350
—. The modified gram stain of Much, 422
KUGEL, MAURICE A. (*See* Rosenthal and Kugel), 344

L

LANDT, HARRY, AND FRIEDMAN, RAYMOND. The dynamic consequences of an aurículopericardial fistula, 1091
LAVIANO, ALICE B. (*See* Gilbert, Coleman, and Laviano), 225
LIGHTNER, ESTELLE LOGAN. (*See* Yardumian and Lightner), 877
LOONEY, J. M., AND STRATTON, F. C. The preparation and use of colloidal carbon solutions, 996
LUKENS, MARGUERITE. (*See* Boerner and Lukens), 1007

M

MACHT, DAVID I., AND PAULSON, MOSES. Phytopharmacology of stomach washings in various digestive disorders and pernicious anemia, 155
MACNEAL, HERBERT P. A dropper for performance of fragility test of red blood corpuscles, 1009
—. Sedimentation time of blood, 1010
MACQUIDDY, E. L. (*See* Myers, MacQuiddy, and Baker), 462, 468
MADDEN, BERNARD. (*See* Kolmer, Rule, and Madden), 972
MAGERS, ELIZABETH J. A comparison of blood sugar curves when glucose is given orally and intraduodenally, 608
MAHER, CHAUNCEY C. (*See* Hines and Maher), 232

MANLY, LEONARD S., AND SAELHOF, CLARENCE C. An improved method of blood culture, 203
MARSDEN, CHARLES S., JR. (*See* Pickard and Marsden), 395
MARSHALL, M. S. (*See* Shevky, Kohl, and Marshall), 689
MATHEWS, W. R. (*See* Hargrove and Mathews), 126
MCCARTY, SARAH H. Elliptical red blood cells in man, 612
MCCLELLAN, R. H., AND ENTWISLE, R. M. Sickle cell anemia, 507
MCCOY, MARY VAN S. Examination for tubercle bacilli, 885
MCMANUS, MARY A. (*See* Rowe, McManus, and Riley), 923
MCNAMARA, W. L. Multiple tumors, 269
MCNEILL, CLYDE. (*See* Dowden, McNeill, and McNeill), 362
MCNEILL, J. D. (*See* Dowden, McNeill, and McNeill), 362
MEDES, GRACE. (*See* Moen, Medes, and Chalek), 571
MEINICKE, ERNST. A new syphilis reaction, the M.K.R. II in cerebrospinal fluids, 518
MELENEY, FRANK L. (*See* Jern, Howes, and Meleney), 1257
MELENEY, HENRY E. The relative incidence of intestinal parasites in hospital patients in Nashville and in rural Tennessee, 113
MEMBER, SAMUEL. (*See* Bruger, Mirsky, and Member), 474
MINNICH, WILLIAM C. (*See* Stein and Minnich), 24
MIRMAN, JOSEPH M. Moniliasis of the biliary tract, 379
MIRSKY, I. ARTHUR. (*See* Bruger, Mirsky, and Member), 474
MOEN, JOHANNES K., MEDES, GRACE, AND CHALEK, I. The relative effects of diathermy and infection on the plasma proteins, plasma viscosity and suspension stability of the blood in dogs, 571
MOENCH, G. L. Variations of the solubility of the cervical mucus in relation to the menstrual cycle, 358
MOOD, G. MCF. An adaptation of sand filtration to the rapid clearing of heavy bacterial culture media, 195
MOON, V. H., AND KENNEDY, PATRICK J. Changes in blood concentration incident to shock, 295
MORGAN, J. R. E. A metastatic tumor of the tricuspid valve arising in a case of malignant teratoma of the testis, 749
MORRISON, R. W. (*See* Bliss and Morrison), 248
MORTIMER, HAROLD M. (*See* Hick and Mortimer), 1058
MULL, J. W. A simple light filter for microblood sugar determinations, 667
MUNCH, JAMES C. (*See* Wolffe and Munch), 1184
MURDOCK, THOMAS P. (*See* Ferguson, Murdock, and Welch), 1304
MURPHY, DOUGLAS P. Improved drinking fountain for small laboratory animals, 312
MYERS, JOHN T., MACQUIDDY, E. L., AND BAKER, CHARLES P. Sodium ricinoleate, 462, 468

N

NAMMACK, CHARLES H., AND BITTMAN, FLORENCE R. A typhoid-like infection associated with an organism resembling bacillus proteus pseudovaleriae (De Assis), 1094
NASH, THOMAS P., JR., AND KRAUSS, WILLIAM. A pipette washing device, 94
NEDZEL, A. J. Cocaine poisoning as influenced by diets, 875
NEIGUS, IRWIN. (*See* Hill, Neigus, and Wilhelmj), 1126
NEIMAN, BENJAMIN H. Verrucous aortitis with special regard to aneurysm formation in children, 929
NELSON, VICTOR E. (*See* Keil and Nelson), 1083
NORWOOD, VERNON H. (*See* Robinson and Norwood), 581

O

OCHI, TAKUMI. (*See* KISÉ AND OCHI), 1073
OSGOOD, EDWIN E., AND WILHELM, MABLE M. Reticulocytes, 1129
OWEN, KENNETH A. The use of galactose in differential diagnosis of jaundice, 1311
OWEN, SEWARD E. Small animal metabolism cage, 1135

P

PARKER, FRANCIS P. (*See* Kracke and Parker), 799
PARKINS, WILLIAM M. (*See* Salant, Parkins, and Sheppard), 142
PARTRIDGE, H. M., BOWLES, J. A. C., AND GOLDFEDER, ANNA. Determination of pH of normal and malignant tissues with the glass electrode and vacuum tube null indicator, 1100
PAUL, HENRY. (*See* Heller and Paul), 777
PAULSON, MOSES. (*See* Macht and Paulson), 155
PETERMAN, E. A., AND COOLEY, THOMAS B. Studies on the quantitative estimation of bile pigments, 723
—, AND —. The use of hydrogen peroxide in quantitative estimation of bilirubin in bile, 743
PETERSILIE, HENRY. A simple device to be used in connection with micro-Kjeldahl digestion process, 672
PETERSON, H. H., AND APPLETON, J. T. T., JR. The blood picture in oral infection, 1079
PHILLIPS, FRANCIS J., AND CURTIS, GEORGE M. Blood iodine studies, 896
PICKARD, RAWSON J., AND MARSDEN, CHARLES S., JR. The relation of blood glutathione to the hemoglobin and amount of red cells, 395
PLATO, AARON. (*See* Eisenberg, Wallerstein, and Plato), 315
POE, CHARLES F., AND BAILEY, JOHN F. A study of the Otto test for strychnine, 40
POINDEXTER, HILDRUS A., AND JONES, R. FRANK. Wuchereria (filaria) bancrofti infection in man with an unusual history, 864
POLOWE, DAVID. The specific gravity of the blood in human cancer, 983
POTH, EDGAR J. A modification of Hill's radiopaque mass for the injection of lumina, 1241
PRITHAM, GORDON H., AND ANDERSON, ARTHUR K. A comparison of methods for the determination of uric acid in human, bovine, and avian bloods, 892
PUTNAM, TRACY J. An easily assembled, self-recording perfusion apparatus, 89
—. A simple automatic sharpener for microtome knives, 199

Q

QUIGLEY, J. P. An automatic-filling ink recording system and permanent type water manometer, 1342
QUINAN, CLARENCE. Pigment studies, 951, 954

R

RAFSKY, HENRY A. Crystalline elements in the stomach lavage of patients with cholelithiasis, 959
RALLI, ELAINE P. (*See* Brown and Ralli), 1169
RANDOLPH, HOWELL. The toxin of bacillus proteus, 870
RATHMELL, THOMAS K., AND CROCKER, WALTER J. The velocity factor in blood transfusion, 1206
RAWLS, WILLIAM B., GRUSKIN, BENJAMIN J., RESSA, ANTHONY A., AND JORDON, MARIE. The sedimentation rate and polymorphonuclear count in rheumatoid and mixed arthritis, 830

RESSA, ANTHONY A. (*See* Rawls, Gruskin, Ressa, and Jordon), 830
RICE, THURMAN B., AND SMITH, VIRGINIA P. A study of the Eagle antigens for Wassermann and flocculation tests, 84
RIDER, T. H. The stability of diothane solutions, 771
RILEY, GERTRUDE A. (*See* Rowe, McManus and Riley), 923
ROBERTS, GEORGE H. (*See* Abramson, Crawford, and Roberts), 1040
ROBERTSON, WILLIAM EGBERT. Alpha dinitrophenol and its influence upon metabolism, 1280
ROBINSON, WILLIAM, AND NORWOOD, VERNON H. Destruction of pyogenic bacteria in the alimentary tract of surgical maggots implanted in infected wounds, 581
—, AND SIMMONS, S. W. Effects of low temperature retardation in the culture of sterile maggots for surgical use, 683
—, AND —. Surgical maggots in the treatment of infected wounds; recent apparatus and methods in maggot production and research, 339
ROEHM, RICHARD R. (*See* Greene and Roehm), 1116
ROHDENBURG, G. L., AND SCHLEUSSNER, R. The light filtering index of blood serum, 705
ROSENTHAL, NATHAN, AND KUGEL, MAURICE A. Hypoleucocytic angina, 344
ROWE, ALLAN WINTER, MCMANUS, MARY A., AND RILEY, GERTRUDE A. The toxemias of pregnancy, 923
RUBINSTEIN, H. S. The inactivation of growth hormone, 63, 404
RUDISILL, HILLYER, JR., AND SHUTE, EVAN. The mechanics of spleen visualization by means of metallic compounds, in particular thorium, 511
RUDY, ABRAHAM. Studies of the physiologic and pathologic chemistry of the skin, 654
RULE, ANNA M. (*See* Kolmer, Rule, and Madden), 972
RUSSELL, E. R. (See Abernethy, Russell, and Thienes), 1014

S

SACKETT, DE LESTER, AND ESELIUS, ERIC. A comparison of Wassermann and Kahn reactions upon spinal fluid in treated and untreated cases of paresis, 546
—, AND TURNER, A. WILLARD. The effect of hyperpyrexia upon the phosphorus partition of whole blood in paresis, 1045
SAELHOF, CLARENCE C. (*See* Manly and Saelhof), 203
SALANT, WILLIAM, PARKINS, WILLIAM M., AND SHEPPARD, LILLIAN E. A study of blood calcium after the administration of sodium oxalate to normal and thyroparathyroidectomized cats, 142
SCHLEUSSNER, R. (*See* Rohdenburg, and Schleussner), 705
SCOTT, ALBERT L. Comparison of the Kolmer-Wassermann, Kahn, and Rosenthal tests for syphilis, 1337
SCOTT, L. D. A new colorimetric method for the determination of biliary acids in body fluids, 523
SEEVERS, M. H. Morphine pentobarbital anesthesia for dog surgery, 202
SENTURIA, BEN D. Glutathione content of blood in chronic arthritis and rheumatoid conditions, 1151
SHAPIRO, CLARA S. A direct nesslerization method for urea nitrogen in blood where commercial urease is employed, 659
SHAW, FREDERICK W. The growth of pathogenic bacteria in immune blood, 77
SHEARD, CHARLES. (*See* Davis and Sheard), 593
SHEPPARD, LILLIAN E. (*See* Salant, Parkins, and Sheppard), 142
SHEVKY, M., KOHL, C., AND MARSHALL, M. S. Bacterium melaningogenicum, 689
SHORT, JAMES J. (*See* Johnson and Short), 962
SHTERNOV, VLADIMIR A. (*See* Klarmann, Shternov, and Gates), 835
SHUTE, EVAN. (*See* Rudisill and Shute), 511
SILER, VINTON E. (*See* Guest and Siler), 757
SIMMONS, S. W. (*See* Robinson and Simmons), 339, 683
SIMONDS, J. P. (*See* Jergesen and Simonds), 1054
SMALL, JAMES C. The treatment of rheumatic carditis with aqueous extracts of streptococci, 695
SMITH, VIRGINIA P. (*See* Rice and Smith), 84
SPECTOR, H. I. The Treatment of bronchopulmonary suppuration of fusospirochetal etiology with small doses of neosalvarsan, 66
SPRAY, ROBB SPALDING. A simple rotary inoculating table, 668
STEIN, DAVID, AND MINNICH, WILLIAM C. Functional studies of patients on antiarthritic medication, 24
STEUER, LEONARD G. Embolism and thrombosis of large branches of the pulmonary artery in heart disease, 265
STOWE, W. P. A simple technic for finding coccidioides immitis, 1013
STRATTON, F. C. (*See* Looney and Stratton), 996
STRAYER, J. W. (*See* Crimm and Strayer), 966
SWANSON, EDWARD E. Studies in the pharmacology of local anesthetics, 120
SWEZY, OLIVE. Some pitfalls in the study of sex hormones, 561 (*E*)

T

TAPKE, ROBERT J. (*See* Herrmann, Ames, and Tapke), 411
THAMANN, FREDERICK. (*See* Kehoe and Thamann), 178
THIBAUDEAU, A. A. The preparation and staining of frozen sections, 204
THIENES, C. H. (*See* Abernethy, Russell, and Thienes), 1014
TSUCHIYA, H. Further studies on cultivation of endameba histolytica and a complement fixation test for amebiasis, 495
TURNER, A. WILLARD. (*See* Sackett and Turner), 1045

V

VAN DE ERVE, J. (*See* Gower and Van de Erve), 81
—. (*See* Hemingway, van de Erve, and Booth), 738
VAUGHAN, WARREN T. Air-borne molds, 111 (*E*)
—. Epidemiology of amebic infection, 795 (*E*)
—. Erythrocyte diameters, 335 (*E*)
—. Food Allergy as a common problem, 53
—. Hazards of statistical interpretation, 914 (*E*)
—. Hyperinsulinism, 222 (*E*)
—. Origin of syphilis, 448 (*E*)
—. Sulphydryl and cellular multiplication, 680 (*E*)

W

WALKER, VIRGINA M. Simple method of drying pipettes, 324
WALLERSTEIN, HARRY. (*See* Eisenberg and Wallerstein), 713
—. (*See* Eisenberg, Wallerstein, and Plato), 315
WALTERS, ORVILLE S. Normal erythrocyte, hemoglobin and packed cell volume standards in young men, 851
WELCH, HENRY. (*See* Ferguson, Murdock, and Welch), 1304
WILCOX, CLARE. (*See* Bullowa and Wilcox), 1156
WILHELM, MABLE M. (*See* Osgood and Wilhelm), 1129

WILHELMJ, CHARLES M. (*See* Hill, Neigus, and Wilhelmj), 1126

WILLIAMS, EDWARD F., JR., ABRAMOWITZ, E. WILLIAM, AND KILLIAN, JOHN A. The value of the monkey for the study of the laxative activity of phenolphthalein, especially in comparing different samples of the drug, 1213

WILLIAMS, JOHN R., AND CHALLIS, FRANK E. Methylene blue as an antidote for aniline dye poisoning, 166

WILLIAMS, ROGER J. (*See* Woodward, Kingery, and Williams), 1216

WISHNOFSKY, MAX. Absorption of dextrose from the human gastrointestinal tract, 1286

WHITLEY, ONA R. Corynebacterium diphtheriae gravis found in Maryland, 943

WOLFFE, JOSEPH B., AND MUNCH, JAMES C. VII. Autonomic imbalance in animals, 1184

WOMACK, N. A. (*See* Cole and Womack), 540

WOODWARD, GLENN J., KINGERY, LYLE B., AND WILLIAMS, ROGER J. The fungicidal power of phenol derivatives, 1216

WRIGHT, CHARLES I. A method of damping the Krogh type of spirometer for studying the respiration of rabbits, 1334

Y

YARDUMIAN, K., AND LIGHTNER, ESTELLE LOGAN. Nasopharyngeal flora and some remarks as to their relation to common colds, 877

YOUNG, A. M. Five hundred and nineteen voge bromine tests of urine for pregnancy, 153

—. Observations on the technic of the Friedman test for pregnancy, 1224

Z

ZIMMERMAN, L. M., GAULT, J. T., HALPERN, S. S., AND DE TAKATS, G. The effect of salyrgan and x-ray on the rate of disappearance of thrombophlebitic edema, 243

SUBJECT INDEX

Abstracts are indicated by (Abst.) after the page number; book reviews, by (B. Rev.) after the page number.

A

Absorption of dextrose from the human gastrointestinal tract, 1286
Abstracts, 101, 211, 327, 439, 555, 674, 790, 905, 1021, 1138, 1246, 1345
Acetanilid, influence of caffeine on effects of, 909 (Abst.)
Acid filtrate, sulphosalicylic—note on the determination of blood urea by direct nesslerization of a sodium tungstate, 783
Acidity, gastric, in thyroid dysfunction, 908 (Abst.)
Actinomycetes, modified Sabouraud medium for cultivation of acid-fast, 910 (Abst.)
Adapter, new microscope for hand spectroscope, 755
Adenoma, benign, of sweat glands, 586
Adolescent male, a study of the red blood cell count and hemoglobin in, 172
Agglutination, granular and flocculer types of, with B. typhosus, a study of, 225
H & O following typhoid vaccination and in an unselected group of individuals, 327 (Abst.)
technic applied to B. pertussis, 558 (Abst.)
Agglutinin content of blood following typhoid and paratyphoid immunization, 445 (Abst.)
Agglutinins, developments in study of, 1144 (E)
Agranulocytosis, etiology of, 799
in childhood, 213 (Abst.)
Air borne molds, 111 (E)
Aleucemic myelosis, 1022 (Abst.)
Alimentary tract, pyogenic bacteria in, of surgical maggots implanted in infected wounds, destruction of, 581
sodium ricinoleate action in, an attempt to determine, 462
Allergic skin reaction in brucella infection, a study of the opsonocytophagic power of the blood and, 1024 (Abst.)
Allergy, food, as a common problem, 53
recent immunologic studies in hypersensitivity to tobacco, 793 (Abst.)
Alpha dinitrophenol and its influence upon metabolism, 1280
Ambulatory patients with auricular fibrillation, a study of quinidin effects on, 232
Amebiasis, complement fixation test for, further studies on cultivation of Endameba histolytica and, 495
Amebic dysentery, unusual experience with, 792 (Abst.)
infection, epidemiology of, 795 (E)
American Indian, medicine of, 1
Amylase, salivary, in patients with pernicious anemia, a study of, 504
Amytal, intravenous lethal doses of, in dog and rabbit and a table of animal dosages compiled from the literature, 1191
Anatomy of Rhesus monkey, 912 (B. Rev.)
Anemia, aplastic, 1139 (Abst.)
analysis of so-called, 1023 (Abst.)
chronic idiopathic hypochromic, 908 (Abst.)
etiology and treatment of, in pregnancy, 1249 (Abst.)
macrocytic in disease of liver, 1022 (Abst.)
nutritional, of rat, effect of various colloidal and crystalloidal metallic compounds in, 1083
of pregnancy, 1358 (E)
pernicious, salivary amylase in patients with, a study of, 504
production of erythrocytes, reticulocytes, and hemoglobin in, 967 (Abst.)
sickle-cell, 507
with autopsy finding, a fatal case of, 126
Anesthesia, morphine pentobarbital, for dog surgery, 202
Anesthetics, local, Diazo reaction for detection of certain, in urine and in tissues, 1018
pharmacology of, studies in, 120
Aneurysm formation in children, verrucous aortitis with special regard to, 929
Angina, hypoleucocytic, 344
Anilin dye poisoning, methylene blue as an antidote for, 166
Animal metabolism cage, small, 1135
Antiarthritic medication, functional studies of patients on, 24
Anticoagulants, changes in cell volume produced by varying concentrations of different, 777
Antidote for anilin dye poisoning, methylene blue as an, 166
Antigen, Eagle, a study of, for Wassermann and flocculation tests, 84
for Henry's reaction, a new, 1023 (Abst.)
Antiseptic dye, penetration of an, demonstration of, into tissues of genitourinary tract, 735
Antisheep hemolysin, production of, a new method for, 666
Antiserums, refining of, 1324
Aortitis, verrucous, with special regard to aneurysm formation in children, 929
Aplastic anemia, 1139 (Abst.)
analysis of so-called, 1023 (Abst.)
Apparatus, combination condensing and receiving vessel, 1116
dog-operating table, 1117
electrical tool, a simple and efficient, for sealing museum jars, 197
for continuous administration of fluid intravenously, 758
for determination of metabolic rate in guinea pigs, a simple and inexpensive, 540
perfusion, an easily assembled, self-recording, 89
Aqueous extracts of streptococci, treatment of rheumatic carditis with, 695
Arterial blood in cancer, reaction of, 909 (Abst.)
Artery, pulmonary, in heart disease, embolism and thrombosis of large branches of, 265
Arthritis, chronic, glutathione content of blood in, and rheumatoid conditions, 1151
rheumatoid, 1142 (Abst.)
and mixed, sedimentation rate and polymorphonuclear count in, 830
Ascites, drainage of, observations on vascular response to, 948
Auricular fibrillation, ambulatory patients with, a study of quinidin effects on, 232
Auriculopericardial fistula, dynamic consequences of, 1091
Automatic and graphic method of recording diuresis, 81
Automatic-filling ink recording system and permanent type water manometer, 1342
Autonomic imbalance in animals, 1184
Autopsy stand for mice, a simple, 210

B

Bacilli, tubercle, routine culture of urine for, 675 (Abst.)
typhoid, isolation of, from water and sewage, 559 (Abst.)
Bacillus abortus, guinea pigs inoculated with, intracutaneous reactions induced in, 589
coli hemolyticus, clinical significance of, 1024 (Abst.)
pertussis, rapid agglutination on technic applied to, 558 (Abst.)

Bacillus, Cont'd
proteus pseudovaleriae (De Assis), a typhoid-like infection associated with an organism resembling, 1094
toxin of, 870
tuberculosis, inhibitory effect of normal blood on growth of, at incubator temperature, 679 (Abst.)
viability and virulence of old cultures of, 790 (Abst.)
typhosus, granular and floccular types of agglutination with, a study of, 225
Bacteria, behavior of bacteriophage and, in lesion after treatment of acute staphylococcus skin infections with bacteriophage, 1257
pathogenic, in immune blood, growth of, 77
pyogenic, in the alimentary tract of surgical maggots implanted in infected wounds, destruction of, 581
Bacterial culture media, clearing of heavy, an adaptation of sand filtration to rapid, 195
endocarditis, subacute, streptococci that resemble diphtheroids recovered from blood cultures in, significance of, 1304
filtration, simplifying the mechanics of, 786
Bactericidal and fungicidal action of homologous halogen phenol derivatives and its "quasi-specific" character, 835
Bacteriologic incubator, an improvised, 1098
Bacteriology, manual of determinative, 1353 (B. Rev.)
of veterinary, 221 (B. Rev.)
textbook of, 1354 (B. Rev.)
Bacteriophage, studies in, 1257
Bacterium melaningogenicum, 689
Barnacles, 1355 (E)
Basal metabolism of old people, 1073
Benign adenoma of the sweat glands, 586
Bicycle ergometer, recording electrodynamic brake, 1105
Bile, bilirubin in, quantitative estimation of use of hydrogen peroxide in, 743
constituents of, a method for the separation of the principal, 1003
pigments, studies on quantitative estimation of, 723
salts, combined effects of, and oleic acid on choleresis, 567
Biliary acids in body fluids, a new colorimetric method for the determination of, 523
tract, diagnostic methods and metabolic studies in disease of, 215 (Abst.)
monilíasis of, 379
Bilirubin, concentration and precipitation of, in the gallbladder and bile ducts, 327 (Abst.)
in bile, quantitative estimation of, use of hydrogen peroxide in, 743
plasma, estimation of, 1122
spectrophotometric investigation and determination of, 593
Biochemistry, practical methods in, 1354 (B. Rev.)
Biologic specimens, preservation of, 774
Biopsy, present status of, 560 (Abst.)
Blood, agglutinin content of, following typhoid and paratyphoid immunization, 445 (Abst.)
and urine, chloral hydrate in, quantitative determination of, 1332
arterial, reaction of, in cancer, 909 (Abst.)
calcium after the administration of sodium oxalate to normal and thyroparathyroidectomized cats, a study of, 142
catalase in heart disease, 133
cell and hemoglobin relations as determined by iron and oxygen capacity methods, 886
count, red, and hemoglobin in the adolescent male, a study of, 172
white, dilution pipettes, the calibration of, 95
cells in, determination of volume of, centrifuge method for, 757
chemistry, nonprotein nitrogen studies and their prognostic significance in acute coronary occlusion, 441 (Abst.)
cholesterol in chronic nephritis and retention uremia, 906 (Abst.)
Blood, Cont'd
concentration incident to shock, changes in, 295
corpuscles, red, a dropper for performance of fragility test of, 1009
count, Schilling differential, in tropical diseases, 644
culture, an improved method of, 203
in tuberculosis, 1021 (Abst.)
cultures, comparative study of, taken with Kendall and routine mediums, 10[illegible] (Abst.)
in subacute bacterial endocarditis, significance of streptococci that resemble diphtheroids recovered from, 1304
cytology in treated and untreated syphilis, 1023 (Abst.)
during first year of life, 791 (Abst.)
fibrinogen, in quantitative determination of, 906 (Abst.)
following glucose ingestion, variations of urea, total nonprotein nitrogen and chloride concentration in, 474
glutathione content of, in chronic arthritis and rheumatoid conditions, 1151
relation of, to hemoglobin and amount of red cells, 395
in normal pregnancy, 1142 (Abst.)
immune, growth of pathogenic bacteria in, 77
inhibitory effect of normal, on growth of B. tuberculosis at incubator temperature, 679 (Abst.)
iodine studies, 818, 896
iron and hemoglobin, a clinical study of, 362
in human, 212 (Abst.)
is halometry reliable, 1351 (Abst.)
lipase in patients with peptic ulcer, 1054
lipids in children with scarlet fever and rheumatic disease, 1246 (Abst.)
lipoid content of, observations upon the Lipokrit method for the determination of, 411
normal, presence of biliary acids in, a note on the alleged, 523
pellagra, a chemical peculiarity of, 329 (Abst.)
picture, 333 (B. Rev.)
in oral infection, 1079
plasma proteins, plasma viscosity and suspension stability of, in dogs, relative effects of diathermy and infection on, 571
proteins, a micromethod for quantitative estimation of, 555 (Abst.)
red cells in man, elliptical, 612
sedimentation, interrelation of Cutler, Linzenmeier and Westergren tests, 1140 (Abst.)
time of, 1010
serum, light filtering index of, 705
of normal children, concentration of calcium and phosphorus of, 679 (Abst.)
tryptophane content of, 1113
specific gravity of, in human cancer, 983
stain, Wright's, modified technic for making, 438
studies in hyperthyroidism, 371
in normal pregnancy, 1248 (Abst.)
study of a lymphocytic hemogram, 330 (Abst.)
sugar curves, comparison of, when glucose is given orally and intraduodenally, 608
in schizophrenia, 792 (Abst.)
transfusion, velocity factor in, 1206
urea, determination of, by direct nesslerization of a sodium tungstate—sulphosalicylic acid filtrate, 783
nitrogen in, direct nesslerization method for, where commercial urease is employed, 659
uric acid in human, bovine, and avian, a comparison of methods for determination of, 892
whole, cholesterol in, a simplified microdetermination of, serum and plasma, 883
in paresis, effect of hyperpyrexia upon phosphorus partition of, 1045
Body fluids, biliary acids in, a new colorimetric method for the determination of, 523
cytologic study of, 315

Book reviews, 108, 220, 333, 447, 911, 1026, 1352
Brain, ethyl alcohol in, estimation of, 1014
Breast, carcinoma of, bases for histologic grading of, 677 (Abst.)
Bright's disease, fractional phenolsulphonephthalein test in, 331 (Abst.)
Bronchopulmonary suppuration of fusospirochetal etiology with small doses of neosalvarsan, treatment of, 66
Brucella infection, allergic skin reaction in, a study of the opsonocytophagic power of the blood and, 1024 (Abst.)

C

Cage for mice and rats, 1020
metabolism, for rats, 669
small animal metabolism, 1135
Calcium and phosphorus, concentration of, in blood serum of normal children, 679 (Abst.)
metabolism and calcium therapy, 108 (B. Rev.)
serum, in normal boys, 440 (Abst.)
Calibration of white blood cell dilution pipettes, 95
Cancer, human, specific gravity of blood in, 983
reaction of arterial blood in, 909 (Abst.)
Carbohydrate tolerance, effect of gain in weight on, 1169
Carcinoma, breast, bases for histologic grading of, 677 (Abst.)
of pancreas, 1058
primary, of lung, 676 (Abst.)
Cardiovascular system or kidneys, serum proteins in diseases not primarily affecting, 1348 (Abst.)
Carditis, rheumatic, treatment of, with aqueous extracts of streptococci, 695
Catalase, blood, in heart disease, 133
Catarrhal jaundice, liver function in, 791 (Abst.)
Cell morphology, study of, a differential stain that advances, 1247 (Abst.)
volume, changes in, produced by varying concentrations of different anticoagulants, 777
packed, normal erythrocyte, and hemoglobin standards in young men, 851
Cells in blood, determination of volume of, centrifuge method for, 757
Cellular multiplication, sulphydryl and, 680 (E)
Central nervous systems, histopathology of the peripheral and, 220 (B. Rev.)
Centrifuge method for the determination of the volume of cells in blood, 757
Cerebrospinal fluids, M.K.R. in, a new syphilis reaction, 518
Cervical mucus, variations of solubility of, in relation to the menstrual cycle, 358
Chemistry of the skin, studies of physiologic and pathologic, 654
Chemotherapeutic studies with sodium ricinoleate (soricin), 972
Children, aneurysm formation in, verrucous aortitis with special regard to, 929
pneumonia in, in Egypt, the causative organisms of, 751
Chloral hydrate, quantitative determination of, in blood and urine, 1332
Chloramines, use of, for disinfection of swimming pools, some factors involved in, 1346 (Abst.)
Chloride concentration in blood following glucose ingestion, variations of urea, total nonprotein nitrogen and, 474
Chlorophyll als Pharmakon, 911 (B. Rev.)
Cholelithiasis, stomach lavage of patients with, crystalline elements in, 959
Choleresis, combined effects of bile salts and oleic acid on, 567
Cholesterol, blood, in chronic nephritis and retention uremia, 906 (Abst.)
simplified microdetermination of, in whole blood, serum, and plasma, 883
Chronic myelosis in children, 713
Cisternal drainage, pneumococcic meningitis with recovery following, report of a case of, 1139 (Abst.)
Coccidia and coccidiosis, 1352 (B. Rev.)
Coccidioides immitis, a simple technic for finding, 1013
Cold, common, 333 (B. Rev.)
Colds, common, nasopharyngeal flora and some remarks as to their relation to, 877
Colloidal and crystalloidal metallic compounds in nutritional anemia of rat, effect of various, 1083
carbon solutions, preparation and use of, 996
gold test, Lange, a modification of, 1007
lead compounds, 178
Colon, irritable, treatment of, with sodium ricinoleate, 234
Colorimetric method for the determination of biliary acids in body fluids, a new, 523
rapid, for multiple determinations of uric acid, 650
Complement fixation test for amebiasis, further studies on cultivation of Endameba histolytica and, 495
Condenser, reflux, a simple and efficient, 314
Corn component of a rachitogenic diet, variability in, 390
Coronary blood supply, to experimentally produced ventricular lesions, relationship between, and resulting electrocardiographic alterations, 1040
occlusion, acute, serial nonprotein nitrogen studies and their prognostic significance in, 441 (Abst.)
and sudden death, 1272
Corpuscles in health and disease, partition of potassium between serum and, 633
Correspondence, 451, 1360
Corynebacterium diphtheriae gravis found in Maryland, 943
Cranial nerves, outline of, 108 (B. Rev.)
Crystalline elements in the stomach lavage of patients with cholelithiasis, 959
Crystalloidal and colloidal metallic compounds in nutritional anemia of rat, effect of various, 1083
Culture medium, differential, for B. diphtheriae and B. pseudodyphtheriae, 439 (Abst.)
Cytologic study of body fluids, 315

D

Dermatitis, contact, patch test in diagnosis of, 555 (Abst.)
Dermatology, history of, 447 (B. Rev.)
Device, pipette washing, 94
Dextrose, absorption of, from the human gastrointestinal tract, 1286
Differential blood count, Schilling, in tropical diseases, 644
Diabetes, insulin resistance in, 1021 (Abst.)
mellitus, characteristics and trends of diabetes mortality throughout world, 678 (Abst.)
mineral water in management of, experiments in use of, 1088
use of synthalin, 1067
mortality throughout world, characteristics and trends of, 678 (Abst.)
Diathermy and infection, relative effects of, on plasma proteins, plasma viscosity and suspension stability of blood in dogs, 571
Diazo reaction for detection of certain local anesthetics in urine and in tissues, 1018
Dietary treatment of undernutrition, 1169
Diets influencing cocaine poisoning, 875
Differential diagnosis of jaundice, use of galactose in, 1311
Digestion process, microKjeldahl, a simple device to be used in connection with, 672
Digestive disorders, phytopharmacology of stomach washings in various, and pernicious anemia 155
Diothane solutions, stability of, 771
Diphtheria, active immunization against, by a single injection, 102 (Abst.)
differential culture medium for B. diphtheriae and B. pseudodyphtheriae, 439 (Abst.)
precipitated toxoid as an immunizing agent against, 1021 (Abst.)
Diuresis, method of recording, an automatic and graphic, 81
Diuretics, xanthine, comparative study of certain, 248

Dog-operating table, 1117
Drainage of ascites, observations on vascular response to, 948
Drinking fountain, improved, for small laboratory animals, 312
Dropper for performance of fragility test of red blood corpuscles, 1069
Duodenal pouches, a method of preparing, for use in experimental work, 1126
Dysentery, amebic, unusual experience with, 792 (Abst.)

E

Eagle antigens for Wassermann and flocculation tests, a study of, 84
Edema, thrombophlebitic, effect of salyrgan and x-ray on rate of disappearance of, 243
Editorial. Anemia of pregnancy, 1358
Barnacles, 1355
Developments in the study of agglutinins, 1144
Epidemiology of amebic infection, 795
Erythrocyte diameters, 335
Have hospitals attained a standard of mediocrity, 1251
Hazards of statistical interpretation, 944
Hyperinsulinism, 222
Laboratory diagnosis of undulant fever, 1029
Origin of syphilis, 448
Some pitfalls in the study of sex hormones, 561
Sulphydryl and cellular multiplication, 680
Eggs, nutrient quality of, for growing tubercle bacilli, 440 (Abst.)
Egypt, pneumonia in children in, causative organisms of, 751
Electrode for measurement of potentials on the human body, an improved, 1234
Electrodynamic brake bicycle ergometer, the recording, 1105
Embolism and thrombosis of large branches of the pulmonary artery in heart disease, 265
Endameba histolytica, cultivation of, further studies on, and a complement fixation test for amebiasis, 495
Endo medium, nature of reaction of colon organism on, 442 (Abst.)
Endocarditis, subacute bacterial significance of streptococci that resemble diphtheroids recovered from blood cultures in, 1304
Enteritis, acute, in infants and young children, 1248 (Abst.)
polytropous, 909 (Abst.)
Eosinophilia, induction of, in normal animals, 909 (Abst.)
reflex, 486
Epidemiology of amebic infection, 795 (E)
of pneumonia, 675 (Abst.)
of syphilis, history and, 447 (B. Rev.)
Epithelial cysts of the skin tissue, traumatic, 1250 (Abst.)
Ergebnisse der medizinischen Strahlenforschung, 1027 (B. Rev.)
Ergometer, recording electrodynamic brake bicycle, 1105
Erysipeloid among workers in a bone button factory due to the bacillus of swine erysipelas, 1021 (Abst.)
Erythrocyte diameters, 335 (E)
normal, hemoglobin and packed cell volume standards in young men, 851
production of, reticulocytes, and hemoglobin in anemia, 907 (Abst.)
Ethyl alcohol in brain estimation of, 1014
Experimental studies on nembutal (pentobarbital-sodium), 738

F

Feces, a convenient specimen container, 328 (Abst.)
Fermentation tubes, Smith and "I" tubes, special rack for, 553
Fibrinogen, quantitative determination of, in blood, 906 (Abst.)
Filter, light, for microblood sugar determinations, 667
Filterable virus diseases in man, 221 (B. Rev.)
Filtering index, light, of blood serum, 705
Filtrate sulphosalicylic acid—note on the determination of blood urea by direct nesslerization of a sodium tungstate, 783
Filtration, bacterial, simplifying the mechanics of, 786
Fistula, auriculopericardial, dynamic consequences of, 1091
Flocculation test, study of Eagle antigens for Wassermann test and, 84
Vernes, in tuberculosis, 905 (Abst.)
tests for syphilis, 558 (Abst.)
Fluid, spinal, sugar determinations in experimental hypoglycemia of dogs, 1049
Food allergy as a common problem, 53
Food-borne infections and intoxications, 913 (B. Rev.)
Fragility test of red blood corpuscles, a dropper for performance of, 1069
Friedman rabbit ovulation test in differential obstetric diagnosis, 1033
test for pregnancy, observations on the technic of, 1224
Frozen sections, preparation and staining of, 204
Functional studies of patients on antiarthritic medication, 24
Fungicidal power of phenol derivatives, 1216
Fusospirochetal disease, oral spirochetes and related organisms in, 109 (B. Rev.)
etiology with small doses of neosalvarsan, treatment of bronchopulmonary suppuration of, 66

G

Galactose in differential diagnosis of jaundice, use of, 1311
Gallbladder and bile ducts, bilirubin in, concentration and precipitation of, 327 (Abst.)
Gastric acidity in thyroid dysfunction, 908 (Abst.)
examination in pulmonary tuberculosis with negative sputum examination, 441 (Abst.)
Gastroenterology, modern aspects of, 221 (B. Rev.)
Gastrointestinal tract, absorption of dextrose from the human, 1286
Genitourinary tract, penetration of an antiseptic dye into tissues of, demonstration of, 735
Glucose, given orally and intraduodenally, comparison of blood sugar curves when, 608
ingestion, blood following, variations of urea, total nonprotein nitrogen, and chloride concentration, 474
Glutathione content of blood in chronic arthritis and rheumatoid conditions, 1151
Gold test, Lange colloidal, a modification of, 1007
Gonococci, cultivation of, 557 (Abst.)
preparation of correct picture and simple culture method of, 1139 (Abst.)
Gram property of acid-fast form of tubercle bacillus, 350
stain of Much, modified, 422
Granular and flocculer types of agglutination with B. typhosus, a study of, 225
Granuloma inguinale, further studies on etiology of, 910 (Abst.)
Granulopenia, etiology of, 799
Growth hormone, the inactivation of, 63
Guinea pigs inoculated with B. abortus, intracutaneous reactions induced in, 589
metabolic rate in, simple and inexpensive apparatus for determination of, 540
Gum dammar for use as a mounting media, purification of, 325

H

Halogen phenol derivatives, homologous, bactericidal and fungicidal action of, and its "quasi-specific" character, 835
Handbuch der biologischen Arbeitsmethoden, 1027 (B. Rev.)
der Chemotherapie, 1028 (B. Rev.)
Hay fever, perennial treatment of, 481
Heart disease, blood catalase in, 133
criteria for the classification and diagnosis of, 109 (B. Rev.)
embolism and thrombosis of large branches of pulmonary artery in, 265
stroke volume of, determination of, 994
Hemoglobin and blood cell relations as determined by iron and oxygen capacity methods, 886

Hemoglobin, Cont'd
content of blood in infants, 107 (Abst.)
clinical study of blood iron and, 362
determination of, by iron content method, 406
in the adolescent male, a study of the red blood cell count and, 172
normal erythrocyte and packed cell volume standards in young men, 851
production of, erythrocytes and reticulocytes in anemia, 907 (Abst.)
relation of blood glutathione to, and amount of red cells, 395
Hemogram, lymphocytic, a study of, 335 (Abst.)
Hemolysin, antisheep, production of, a new method for, 666
Hemolytic properties of streptococci mastitis, 1347 (Abst.)
streptococci isolated from hemorrhagic smallpox, studies on, 280
Hemorrhagic smallpox, hemolytic streptococci isolated from, studies on, 280
Hepatic function, levulose and galactose tolerance tests for, clinical values of, 442 (Abst.)
Hill's radiopaque mass for the injection of lumina, a modification of, 1241
Histochemical studies of organs of tumor-bearing rats by the microincineration method 1293
Histopathology of the peripheral and central nervous systems, 220 (B. Rev.)
Hodgkin's disease, a probable unusual case of, 625
Hormone diagnosis of viability of pregnancy, 674 (Abst.)
growth, the inactivation of, 63, 404
Hormones, sex, some pitfalls in the study of, 561 (E)
Hospital routine, demonstration of tubercle bacilli for use in, an evaluation of three methods for, 429
Hospitals attained a standard of mediocrity, have, 1251 (E)
Human body, measurement of potentials on, an improved electrode for, 1234
mind, a view of, and how it works, 220 (B. Rev.)
Hydatidiform mole, differentiation of pregnancy and, 216 (Abst.)
Hydrogen ion concentration, determination of, of normal and malignant tissues with the glass electrode and vacuum tube null indicator, 1160
peroxide, use of, in quantitative estimation of bilirubin in bile, 743
Hydrolyzed adrenalin as a tissue stain, on the importance of, 954
Hyperglycemia, hypertension, obesity and, 441 (Abst.)
Hyperinsulinism, 222 (E)
Hyperplasia, thymic, follow-up study of 30 cases of, 1246 (Abst.)
Hyperpyrexia, effect of, upon phosphorus partition of whole blood in paresis, 1045
Hypersensitivity to tobacco, recent immunologic studies in, 793 (Abst.)
Hypertension, obesity and hyperglycemia, 441 (Abst.)
Hyperthyroidism, blood studies in, 371
Hypoglycemia of dogs, spinal fluid sugar determinations in experimental, 1049

I

Imbalance, autonomic, in animals, 1184
Immune blood, pathogenic bacteria in, growth of, 77
Immunity, an outline of, 1352 (B. Rev.)
Immunization, typhoid and paratyphoid, agglutinin content of blood following, 445 (Abst.)
Incubation, maintaining water and air balance during prolonged, 899
Incubator, an improvised bacteriologic, 1098
Infants, acute enteritis in, and young children, 1248 (Abst.)
pneumonia in newborn and stillborn, 1141 (Abst.)
Infection, oral, blood picture in, 1079
Influenza, with special reference to part played by Pfeiffer's bacillus, streptococci, pneumonococci, and the virus theory, 1026 (B. Rev.)
Inguinal glands in human syphilis, transference of, 1249 (Abst.)
Inoculating table, another simple rotary, 1360 (Correspondence)
simple rotary, 668
Insulin, gauging the dose of, 1173
resistance in diabetes, 1021 (Abst.)
Intestinal flora during oral administration, a study of, 468
parasites, relative incidence of, in hospital patients in Nashville and in rural Tennessee, 113
Intoxications, food-borne infections and, 913 (B. Rev.)
Intracellular structures in monocytes in cases of malignant disease, 917
Intracutaneous reactions induced in guinea pigs inoculated with B. abortus, 589
Intradermal test, Bachman, in human trichinosis, 1350 (Abst.)
in diagnosis of trichiniasis, use of, 1349 (Abst.)
Intravenous administration, continuous, of fluid, a practical method for, 768
lethal doses of amytal in dog and rabbit and table of animal dosages compiled from literature, 1191
vaccination with streptococci, 908 (Abst.)
Iodine content, normal, of human blood, 818
improved micromethod for estimating, 557 (Abst.)
studies, blood, 818, 896
Iron and oxygen capacity methods, hemoglobin and blood cell relations as determined by, 886
content method, determination of hemoglobin by, 406

J

Jaundice, catarrhal, liver function in, 791 (Abst.)
differential diagnosis, use of galactose in, 1311

K

Kahn and Wassermann reactions upon spinal fluid in treated and untreated cases of paresis, a comparison of, 546
Kolmer-Wassermann, and Rosenthal tests for syphilis, comparison of, 1337
reaction in tropics, Kolmer and Meinicke, 780
Kidney, renal function in persons with one, 329 (Abst.)
Kolmer reactions in tropics, Kahn and Meinicke, 780
Kolmer-Wassermann, Kahn, and Rosenthal tests for syphilis, comparison of, 1337
Krebs, seine Entstehung und Erklärung, 911 (B. Rev.)
Krogh type of spirometer for studying the respiration of rabbits, a method of damping, 1334
Kuttner-Lichtenstein microcolorimetric method for the determination of organic phosphorus, 1012

L

Laboratory animals, improved drinking fountain for small, 312
diagnosis of undulant fever, 1029 (E)
Lange colloidal gold test, a modification of, 1007
Lavage, stomach, crystalline elements in, of patients with cholelithiasis, 959
Laxative activity of phenolphthalein, value of the monkey for the study of, especially in comparing different samples of the drug, 1213
Lead, behavior of, in animal organism, 178
poisoning of wild ducks, a study of punctate stippling as found in, 514
Leucemia, monocytic, 1253 (Abst.)
Leucemic reticulo-endiotheliosis, 1250 (Abst.)
Leucocyte count in pulmonary tuberculosis, effect of exercise on, 443 (Abst.)
Leucocytes, unusual hematologic reaction to neoarsphenamine, 675 (Abst.)
Leucopenia, infectious, an unusual form of, 344
Light filter for microblood sugar determinations, a simple, 667
filtering index of blood serum, 705

Lipoid content of blood, observations upon the Lipokrit method for the determination of, 411
Lipokrit method, observations upon, for the determination of the lipoid content of blood, 411
Liver function, an appraisal of the modified dextrose tolerance tests, 105 (Abst.)
galactose tolerance as a measure of, 217 (Abst.)
in advanced pulmonary tuberculosis, 794
in catarrhal jaundice, 791 (Abst.)
urinary test as index of absorption of gallbladder dye when given orally, 219 (Abst.)
macrocytic anemia in disease of, 1022 (Abst.)
silver nitrate reaction and its applicability to diagnosis of diseases of, 905 (Abst.)
Local anesthetics, detection of, in urine and in tissues, Diazo reaction for, 1018
studies in the pharmacology of, 120
Lobar pneumonia, epidemiology of, 1143 (Abst.)
relation of pneumococcus chain formation to prognosis in, 1156
Lumina, Hill's radiopaque mass for injection of, a modification of, 1241
Lung, primary carcinoma of, 676 (Abst.)
Lungs, silica content of, of a group of tunnel workers, 1351 (Abst.)

M

Macrocytic anemia in disease of liver, 1022 (Abst.)
Macropolycyte, 453
Maggot production and research, recent apparatus and methods in, 339
Maggots, sterile, for surgical use, effects of low temperature retardation in culture of, 683
surgical, in treatment of infected wounds, 339
pyogenic bacteria in alimentary tract of, implanted in infected wounds, destruction of, 581
Malaria, method of making slide smears from female anopheles, 556 (Abst.)
new antigen for Henry's reaction, 1023 (Abst.)
vital staining of mitochondria in, 905 (Abst.)
Malignant disease, monocytes in cases of, intracellular structures in, 917
Manometer, permanent type water, an automatic-filling ink recording system and, 1342
Media, bacterial culture, an adaptation of sand filtration to rapid clearing of heavy, 195
Medical dictionary, Stedman's, 1028 (B. Rev.)
secretary, 110 (B. Rev.)
Medicine, clinical, a system of, 912 (B. Rev.)
of the American Indian, 1
preventive, outline of, 109 (B. Rev.)
Mediocrity, standard of, have hospitals attained, 1251 (E)
Meinicke reactions in tropics, Kolmer and Kahn, 780
Meningitis, lactic acid of spinal fluid in, 219 (Abst.)
pneumococcic, report of a case of, with recovery following cisternal drainage, 1139 (Abst.)
Meningococci, rough and smooth variants in stock cultures of, 218 (Abst.)
variation in agglutination of stock cultures of, 218 (Abst.)
Menstrual cycle, variations of solubility of cervical mucus in relation to, 358
Metabolic rate in guinea pigs, a simple and inexpensive apparatus for determination of, 540
Metabolism, alpha dinitrophenol and its influence upon, 1280
basal, of old people, 1073
cage for rats, 669
small animal, 1135
calcium and calcium therapy, 109 (B. Rev.)
nitrogen, 923
Metallic compounds, effect of various colloidal and crystalloidal, in nutritional anemia of rat, 1083
mechanics of spleen visualization by means of, in particular thorium, 511
Metaplastic changes in the prostate gland, 301
Metastatic tumor of tricuspid valve arising in a case of malignant teratoma of testis, 749
Methylene blue as an antidote for anilin dye poisoning, 166
Metycaine, comparison of, with cocaine and procaine on experimental animals, 12
Microblood sugar determinations, a simple light filter for, 667
Microcolorimetric method, Kuttner-Lichtenstein, for the determination of organic phosphorus, note on the procedure in, 1012
Microincineration method, histochemical studies of organs of tumor-bearing rats by, 1293
Micro-Kjeldahl digestion process, a simple device to be used in connection with, 672
Micromethod for estimating iodine, 557 (Abst.)
for quantitative estimation of blood proteins, 555 (Abst.)
Microscope adapter for hand spectroscope, a new, 755
objective, using a "dry," on uncovered objects, 910 (Abst.)
Microtitrations, simple reservoir buret for making, 897
Microtome knives, a simple automatic sharpener for, 199
Milk, brom-thymol-blue reaction in freshly drawn, a study of, 439 (Abst.)
examination of, for members of Escherichia-Aerobacter group, 1143 (Abst.)
preservation of, for streptococcus and abortus examination, 905 (Abst.)
use of glycerol as a preservative for specimens to be examined for hemolytic streptococci, 104 (Abst.)
Mineral water, experiments in use of, in management of diabetes mellitus, 1088
Molds, air-borne, 111 (E)
Moniliasis of the biliary tract, 379
Monocytes, intracellular structures in, in cases of malignant disease, 917
Mononucleosis, infectious: a diagnostic test for, 217 (Abst.)
Morphine pentobarbital anesthesia for dog surgery, 202
Morphology, cell, the study of, differential stain that advances, 1247 (Abst.)
Much, modified Gram stain of, 422
Museum jars, electrical tool for sealing, a simple and efficient, 197
Myelosis, aleucemic, 1022 (Abst.)
chronic, in children, 713

N

Nasopharyngeal flora and some remarks as to their relation to common colds, 877
Nembutal, some experimental studies on, 738
Neosalvarsan, treatment of bronchopulmonary suppuration of fusospirochetal etiology with small doses of, 66
Nephritis, chronic and retention uremia, blood cholesterol in, 906 (Abst.)
types of, and their management, 1348 (Abst.)
Nerves, cranial, outline of, 108 (B. Rev.)
Nesslerization method for urea nitrogen in blood where commercial urease is employed, a direct, 659
of a sodium tungstate, note on determination of blood urea by direct—sulphosalicylic acid filtrate, 783
Neutropenia, benign and malignant, 1350 (Abst.)
Neutrophil in pernicious anemia, 331 (Abst.)
Newborn, sedimentation reaction in, 944
News item, 224
Nitrogen metabolism, 923
total nonprotein, and chloride concentration in blood following glucose ingestion, variations of urea, 474
Normale und pathologische Physiologie der Milz, 911 (B. Rev.)

O

Obesity, hypertension, and hyperglycemia, 441 (Abst.)
Occlusion, coronary, and sudden death, 1272
Oleic acid on choleresis, combined effects of bile salts and, 567

Opsono-cytophagic power of blood and allergic skin reaction in brucella infection, a study of, 1024 (Abst.)
Oral infection, blood picture in, 1079
Organic phosphorus, determination of, Kuttner-Lichtenstein microcolorimetric method for, note on the procedure in, 1012
Organs of tumor-bearing rats, histochemical studies of, by microincineration method, 1293
Otto test for strychnine, a study of, 40
Ovulation test, Friedman rabbit, in differential obstetric diagnosis, 1033
rabbit, for pregnancy, 445 (Abst.)
Oxygen and iron capacity methods, hemoglobin and blood cell relations as determined by, 886
therapy, observations on, 1164

P

Pancreas, carcinoma of, 1058
Parachlorophenol, derivatives of, 835
Parasites, intestinal, relative incidence of, in hospital patients in rural Tennessee, 113
Paresis, spinal fluid in treated and untreated cases of, a comparison of Wassermann and Kahn reactions upon, 546
whole blood in, effect of hyperpyrexia upon phosphorus partition of, 1045
Patch test in diagnosis of contact dermatitis, 555 (Abst.)
ointment, 1138 (Abst.)
Pathogenic bacteria in immune blood, growth of, 77
Pathology, textbook of, 913 (B. Rev.)
Pellagra blood, a chemical peculiarity of, 329 (Abst.)
review of cases with special reference to gastric secretions, 218 (Abst.)
Pentobarbital anesthesia, morphine, for dog surgery, 202
Pentobarbital-sodium (nembutal), some experimental studies on, 738
Pentosuria, studies in, 214 (Abst.)
Peptic ulcer, blood lipase in patients with, 1054
mechanism of pain in, studies of, 47
Perennial treatment of hay fever, 481
Perfusion apparatus, an easily assembled, self-recording, 89
Peritoneal cavity, changes in composition of fluids injected into, 679 (Abst.)
infections, changes in composition of fluids injected into peritoneal cavity, 679 (Abst.)
Pernicious anemia, neutrophil in, 331 (Abst.)
phytopharmacology of stomach washings in various digestive disorders and, 155
salivary amylase in patients with, a study of, 504
Pertussis, occurrence of Bordet-Gengou bacillus, 103 (Abst.)
production of antipertussis serum of high titer, 101 (Abst.)
Pharmacology of local anesthetics, studies in, 120
Phenol derivatives, fungicidal power of, 1216
Phenophthalein, laxative activity of value of the monkey for the study of, especially in comparing different samples of the drug, 1213
Phosphorus and calcium, concentration of, in blood serum of normal children, 679 (Abst.)
organic, determination of, note on the procedure in Kuttner-Lichtenstein microcolorimetric method for, 1012
partition of whole blood in paresis, effect of hyperpyrexia upon, 1045
Photography, invisible screen, 440 (Abst.)
Physikalische Seite des Blutgerinnungs problems un ihre praktische Bedeutung, 1027 (B. Rev.)
Physiologic and pathologic chemistry of the skin, studies of, 654
Phytopharmacology of stomach washings in various digestive disorders and pernicious anemia, 155
Pigment studies, 951, 954
Pipette washing device, 94
Pipettes, calibration of white blood cell dilution, 95
simple method of drying, 324
Plasma bilirubin, estimation of, 1122
cholesterol in whole blood, serum and, a simplified microdetermination of, 883
proteins, effects of diathermy and infection on, relative, plasma viscosity and suspension stability of the blood in dogs, 571
viscosity, relative effects of diathermy and infection on, plasma proteins and suspension of stability of blood in dogs, 571
Pneumonia, causative organisms of, in children in Egypt, 751
epidemiology of, 675 (Abst.)
in infants, due to B. mucosus-capsulatus, 106 (Abst.)
in newborn and stillborn infants, 1141 (Abst.)
lobar epidemiology of, 1143 (Abst.)
relation of pneumococcus chain formation to prognosis in, 1156
significance of newly classified types of pneumococci in disease, 674 (Abst.)
specific serum treatment of Type I, 101 (Abst.)
Type I pneumococcus, value of skin test with type-specific capsular polysaccharide in serum treatment of, 441 (Abst.)
Pneumococci in disease, significance of newly classified types of, 674 (Abst.)
Pneumococcus chain formation, 1156
specific precipitation test for standardization of Type I antipneumococcus serum, 211 (Abst.)
Poisoning, cocaine, as influenced by diets, 875
Polycythemia, benign familial, 560 (Abst.)
Polymorphonuclear count in rheumatoid and mixed arthritis, sedimentation rate and, 830
Potassium, partition of, between serum and corpuscles in health and disease, 633
Potentials, electrode for measurement of, an improved, on the human body, 1234
Precipitate, measurement of volume of, determination of serum protein by, 1320
Pregnancy, anemia in, 1358 (E)
in, etiology and treatment of, 1249 (Abst.)
blood in normal, 1142 (Abst.)
studies, of, in normal, 1248 (Abst.)
Friedman test for, observations on the technic of, 1224
hormone diagnosis of viability of, 105, 674 (Absts.)
rabbit ovulation test for, 445 (Abst.)
sex determination test of Dorn and Sugarman, 1347 (Abst.)
toxemias of, 923
urea clearance test in, 677 (Abst.)
Voge bromine tests of urine for, five hundred and nineteen, 153
Preservation of biologic specimens, 774
Preventive medicine, outline of, 109 (B. Rev.)
Prostate gland, metaplastic changes in, 301
Proteins, blood, micromethod for quantitative estimation of, 555 (Abst.)
serum, diagnostic value of changes in kala azar, 558 (Abst.)
Psychodometer, a simplified, 1238
Puerperal infection, relationship between exogenous throat streptococci and, 104 (Abst.)
Pulmonary artery in heart disease, embolism and thrombosis of large branches of, 265
tuberculosis, advanced, liver function in, 794 (Abst.)
vitamin therapy in, 966
Punctate stippling as found in lead poisoning of wild ducks, a study of, 514
Pyogenic bacteria in alimentary tract of surgical maggots implanted in infected wounds, destruction of, 581

Q

Quantitative determination of chloral hydrate in blood and urine, 1332
estimation of bile pigments, studies on, 723
of bilirubin in bile, use of hydrogen peroxide in, 743
Quinidin effects on ambulatory patients with auricular fibrillation, a study of, 232

R

Rabbit ovulation test, Friedman, in differential obstetric diagnosis, 1033
Radiopaque mass, Hill's, for the injection of lumina, a modification of, 1241
Rachitogenic diet, variability in corn component of a, 390
Rack for Smith fermentation tubes and "U" tubes, 553
Reaction, Diazo, for detection of certain local anesthetics in urine and in tissues, 1018
on a brown skin-adrenalin color, 951
Sachs-Witebsky, for diagnosis of syphilis, value of, 907 (Abst.)
sedimentation in the newborn, 944
Reactions, intracutaneous, induced in guinea pigs inoculated with B. abortus, 589
Kolmer, Kahn, and Meinicke, in the tropics, 780
Recording system, an automatic-filling ink, and permanent type water manometer, 1342
Red Blood cell count and hemoglobin in the adolescent male, a study of, 172
diameters, 333 (B. Rev.)
cells in man, elliptical, 612
corpuscles, fragility test of, a dropper for performance of, 1069
Reflex eosinophilia, 486
Renal function, fractional phenolsulphonephthalein test in Bright's disease, 331 (Abst.)
in persons with one kidney, 329 (Abst.)
tests, normal variations in, 329 (Abst.)
Respiration of rabbits Krogh type of spirometer for studying, a method of damping, 1334
Reticulocytes, 1129
new method of enumeration, 443 (Abst.)
production of, erythrocytes and hemoglobin in anemia, 907 (Abst.)
Reticulo-endotheliosis, leucemia, 1250 (Abst.)
Rheumatic carditis, treatment of, with aqueous extracts of streptococci, 695
disease, blood lipids in children with scarlet fever and, 1246 (Abst.)
fever, intravenous vaccination with streptococci, 908 (Abst.)
Rheumatoid and mixed arthritis, sedimentation rate and polymorphonuclear count in, 830
arthritis, 1142 (Abst.)
conditions, glutathione content of blood in chronic arthritis and, 1151
Ricinoleate, sodium, 462, 468
chemotherapeutic studies with, 972
Rosenthal, Kolmer-Wassermann, and Kahn tests for syphilis, comparison of, 1337
Rotary inoculating table, a simple, 668

S

Salivary amylase in patients with pernicious anemia, a study of, 504
Salmonella suipestifer infection in a child, 678 (Abst.)
in man, 211 (Abst.)
Salyrgan, effect of, and x-ray on the rate of disappearance of thrombophlebitic edema, 243
Sand filtration, an adaptation of, to rapid clearing of heavy bacterial culture media, 195
Scarlet fever and rheumatic disease, blood lipids in children with, 1246 (Abst.)
effect of tonsillectomy on the development of immunity to, as shown by Dick test, 216 (Abst.)
heterologous, 216 (Abst.)
Schilling differential blood count in tropical diseases, 644
Schizophrenia, blood sugar in, 792 (Abst.)
Schultz-Charlton test, 1022 (Abst.)
Secretary, medical, 110 (B. Rev.)
Sedimentation rate and polymorphonuclear count in rheumatoid and mixed arthritis, 830
reaction in the newborn 944
test in dermatology, 215 (Abst.)
time of blood, 1010
Semen, a differential stain that advances the study of cell morphology, 1247 (Abst.)
Serology of syphilis, studies in, 621
Serum and corpuscles in health and disease, partition of potassium between, 633
blood, light filtering index of, 705
tryptophane content of, 1113
calcium, in normal boys, 440 (Abst.)
cholesterol in whole blood, plasma and, a simplified microdetermination of, 883
protein, determination of, by measurement of volume of precipitate, 1320
diagnostic value of changes in kala azar, 558 (Abst.)
in diseases not primarily affecting the cardiovascular system or kidneys, 1348 (Abst.)
test for syphilis, choosing, 275
therapy and vaccine, recent advances in, 1353 (B. Rev.)
Sex determination test of Dorn and Sugarman, 1347 (Abst.)
hormones, some pitfalls in the study of, 561 (E)
Sharpener for microtome knives, a simple automatic 199
Shock, blood concentration incident to, changes in, 295
Sickle cell anemia, 507
with autopsy findings, a fatal case of 126
Silicosis, pathology of so-called acute, 1351 (Abst.)
silica content of lungs of a group of tunnel workers, 1351 (Abst.)
silicon dioxide content of lungs in health and disease, 219 (Abst.)
Silver nitrate reaction and its applicability to diagnosis of diseases of liver, 905 (Abst.)
Skin, chemistry of, studies of physiologic and pathologic, 654
temperature, effect of smoking on, 962
test, intracutaneous, comparison of relative values of, and of pathogen-selective culture in selecting bacteria for vaccines from mixed cultures, 439 (Abst.)
value of, with type-specific capsular polysaccharide in serum treatment of Type I pneumococcus pneumonia, 441 (Abst.)
tissue, traumatic epithelial cysts of, 1250 (Abst.)
Skin-adrenalin color reaction, on a brown, 951
Smallpox, hemorrhagic, hemolytic streptococci isolated from, studies on, 280
Smith fermentation tubes and "U" tubes, special rack for, 553
Smoking, effect of, on skin temperature, 962
Sodium oxalate to normal and thyroparathyroidectomized cats, a study of blood calcium after the administration of, 142
ricinoleate, 462, 468
chemotherapeutic studies with, 972
treatment of irritable colon with, 234
taurocholate and virulence of human tubercle bacilli, 1179
Soricin (sodium ricinoleate), chemotherapeutic studies with, 972
Specific gravity of blood in human cancer, 983
Spectrophotometric investigation and determination of bilirubin, 593
Spectroscope, hand, a new microscope adapter for, 755
Spezifizität der serologischen Reaktionen, 913 (B. Rev.)
Spinal fluid in meningitis, lactic acid of, 219 (Abst.)
sugar determinations in experimental hypoglycemia of dogs, 1049
Wassermann and Kahn reactions upon, a comparison of, in treated and untreated cases of paresis, 546
Spirocheta pallida, a new method of staining, 332 (Abst.)
Spirochetes, oral, and related organisms in fusospirochetal disease, 108 (B. Rev.)
Spirometer for studying the respiration of rabbits, a method of damping the Krogh type of, 1334
Spleen visualization, mechanics of, by means of metallic compounds, in particular thorium, 511
Sputum examination, pulmonary tuberculosis with negative, gastric examination in, 441 (Abst.)

Sputum, Cont'd
number of tubercle bacilli in, a technic for estimating, 328 (Abst.)
tubercle bacilli in, heavy drop method for demonstration of, 445 (Abst.)
Stain, differential, that advances study of cell morphology, 1247 (Abst.)
tissue, hydrolyzed adrenalin as, on importance of, 954
Staining of frozen sections, preparation and, 204
Staphylococcus skin infections, treatment of acute, with bacteriophage, behavior of bacteriophage and bacteria in lesion after, 1257
Statistical interpretation, hazards of, 914 (E)
Stedman's medical dictionary, 1028 (B. Rev.)
Sterile maggots for surgical use, effects of low temperature retardation in culture of, 683
Sterility indicators, use of, studies on rubber glove sterilization and, 382
Stomach lavage, crystalline elements in, of patients with cholelithiasis, 959
washings, phytopharmacology of, in various digestive disorders and pernicious anemia, 155
Streptococci, aqueous extracts of, treatment of rheumatic carditis with, 695
hemolytic, isolated from hemorrhagic smallpox, studies on, 280
properties of mastitis, 1347 (Abst.)
significance of, that resemble diphtheroids recovered from blood cultures in subacute bacterial endocarditis, 1304
Stroke volume of heart, determination of, 994
Strychnine, Otto test for, a study of, 40
Sugar determinations microblood, a simple light filter for, 667
Sulphosalicylic acid filtrate—sodium tungstate, direct nesslerization of, note on the determination of blood urea by, 783
Sulphydryl and cellular multiplication, 681 (E)
Surgery, dog, morphine pentobarbital anesthesia for, 202
Surgical maggots in treatment of infected wounds, 339
pyogenic bacteria in alimentary tract of, implanted in infected wounds, destruction of, 581
pathology, 110 (B. Rev.)
tuberculosis, diagnosis of, 309
use, sterile maggots for, effects of low temperature retardation in culture of, 683
Sweat glands, benign adenoma of, 586
Swimming pools, disinfection of, some factors involved in use of chloramines for, 1346 (Abst.)
Synthalin in diabetes mellitus, use of, 1067
Syphilis, blood cytology in treated and untreated, 1023 (Abst.)
conjugal, a statistical study, 910 (Abst.)
diagnosis of, value of Sachs-Witebsky reaction (citochol) for, 907 (Abst.)
flocculation tests for, 558 (Abst.)
history and epidemiology of, 447 (B. Rev.)
origin of, 448 (E)
reaction, new, M. K. R. II in cerebrospinal fluids, 518
serodiagnosis of, 101 (Abst.)
serology of, studies in, 621
serum test for, choosing, 275
tests for, Kolmer-Wassermann, Kahn, and Rosenthal, comparison of, 1337
transference of inguinal glands in human, 1249 (Abst.)

T

Table, dog-operating, 1117
Temperature retardation, effect of low, in culture of sterile maggots for surgical use, 683
skin, effect of smoking on, 962
Teratoma, malignant, of testis, metastatic tumor of tricuspid valve arising in a case of, 749
Test, complement fixation, for amebiasis, further studies on cultivation of Endameba histolytica and, 495
flocculation and Wassermann, study of Eagle antigens for, 84
for syphilis, Kolmer-Wassermann, Kahn, and Rosenthal, comparison of, 1337
Test, Cont'd
Friedman, for pregnancy, observations on technic of, 1224
rabbit ovulation, in differential obstetric diagnosis, 1033
Lange colloidal gold, modification of, 1007
Schultz-Charlton, 1022 (Abst.)
urea clearance, in pregnancy, 677 (Abst.)
Wassermann and flocculation, study of Eagle antigens for, 84
Testis, malignant teratoma of, metastatic tumor of tricuspid valve arising in case of, 749
Tetanus antitoxin, development of, following administration of tetanus toxoid, 1139 (Abst.)
Thorium compound, mechanics of spleen visualization of means of, 511
Thrombophlebitic edema, effect of salyrgan and x-ray on rate of disappearance of, 243
Thrombosis, femoral, complicated by, New Jersey outbreak of trichinosis, with report of a case, 1350 (Abst.)
of large branches of pulmonary artery in heart disease, embolism and, 265
Thymic hyperplasia, follow-up study of 30 cases of, 1246 (Abst.)
Thyroid disease, blood cholesterol in, effect of treatment on, 104 (Abst.)
dysfunction, gastric acidity in, 908 (Abst.)
Thyroparathyroidectomized cats, a study of blood calcium after administration of sodium oxalate to normal and, 142
Tissue, normal and malignant, determination of pH of, with glass electrode and vacuum tube null indicator, 1100
penetration of an antiseptic dye into, of genitourinary tract, demonstration of, 735
pH of formalin—a factor in fixation, 1024 (Abst.)
skin, traumatic epithelial cysts of, 1250 (Abst.)
stain, hydrolyzed adrenalin as, on importance of, 954
Toxemias of pregnancy, 923
Toxin of bacillus proteus, 870
Transfusion, blood, velocity factor in, 1206
Trichiniasis, diagnosis of, use of an intradermal test in, 1349 (Abst.)
Trichinosis, Bachman intradermal test in human, 1350 (Abst.)
New Jersey outbreak of, with report of case complicated by femoral thrombosis, 1350 (Abst.)
Trichophytin test, report of 350 cases, 213 (Abst.)
Tricuspid valve, metastatic tumor of, arising in a case of malignant teratoma of testis, 749
Tryptophane content of blood serum, 1113
Tubercle bacillus, acid-fast form of, gram property of, 350
bacilli, examination for, 885
experiences gained from growing, 899
for use in hospital routine, an evaluation of three methods for demonstration of, 429
in sputum, heavy drop method for demonstration of, 445 (Abst.)
nutrient quality of eggs for growing, 440 (Abst.)
routine culture of urine for, 675 (Abst.)
virulence of human, sodium taurocholate and, 1179
Tuberculin, ointment patch test, 1138 (Abst.)
test, value of negative intracutaneous (Mantoux) test in adults, 1141 (Abst.)
Tuberculosis, blood culture in, 1021 (Abst.)
effect of vaccination with B. C. G. on children from tuberculous families, 1347 (Abst.)
etiology of, 108 (B. Rev.)
hematological retrospect, 792 (Abst.)
pulmonary, advanced, liver function in, 794 (Abst.)
comparison of blood sedimentation rate and Vernes flocculation test in, 212 (Abst.)
effect of exercise on leucocyte count in, 443 (Abst.)
gastric examination in, with negative sputum examination, 441 (Abst.)

Tuberculosis, pulmonary, Cont'd
question of tubercle bacilli in blood in advanced, 102 (Abst.)
vitamin therapy in, 966
sanocrysin treatment, 444 (Abst.)
surgical, diagnosis of, 309
technic for estimating number of tubercle bacilli in sputum, 328 (Abst.)
tuberculin patch test in, 106 (Abst.)
Vernes flocculation test in, 905 (Abst.)
Tularemia, culture medium for rapid growth of P. tularensis in, 214 (Abst.)
treated by a new specific antiserum, 1141 (Abst.)
Tumor, carcinoid of gastrointestinal tract, 218 (Abst.)
growth and "heavy water," 1139 (Abst.)
metastatic, of tricuspid valve arising in case of malignant teratoma of testis, 749
multiple, 269
Tumor-bearing rats, histochemical studies of organs of, by microincineration method, 1293
Typhoid and paratyphoid immunization, agglutinin content of blood following, 445 (Abst.)
bacilli, isolation of, from water and sewage, 559 (Abst.)
carriers, serological diagnosis of, 442 (Abst.)
vaccination, agglutination, H & O following, and in an unselected group of individuals, 327 (Abst.)
Typhoid-like infection associated with an organism resembling bacillus proteus pseudovaleriae (De Assis), 1094

U

Ulcer, peptic, blood lipase in patients with, 1054
mechanism of pain in, studies of, 47
Uncinariasis, acute from massive infestation and its implications, 560 (Abst.)
Undernutrition, dietary treatment of, 1169
Undernutrition, dietary treatment of, 1169
1345 (Abst.)
laboratory diagnosis of, 1029 (E)
Urea clearance test in pregnancy, 677 (Abst.)
nitrogen in blood, direct nesslerization method for, where commercial urease is employed, 659
variations of, total nonprotein nitrogen and chloride concentration in blood following glucose ingestion, 474
Urease, commercial, is employed, direct nesslerization method for urea nitrogen in blood where, 659
Uremia, retention, blood cholesterol in chronic nephritis and, 906 (Abst.)
Uric acid, determinations of, rapid colorimetric method for multiple, 650
in human, bovine, and avian bloods, comparison of methods for determination of, 892
Urinary test as index of absorption of gallbladder dye when given orally, 219 (Abst.)
Urine and blood, chloral hydrate in, quantitative determination of, 1332
lactose and glucose in, practical method for simultaneous demonstration of, 21[illegible] (Abst.)
local anesthetics in, and in tissues, Diazo reaction for detection of certain, 101[illegible]
routine culture of, for tubercle bacilli, 67[illegible] (Abst.)
Voge bromine tests of, for pregnancy, five hundred and nineteen, 153
Urology, manual of, 333 (B. Rev.)

V

Vaccination, effect of, with B. C. G. on children from tuberculous families, 134[illegible] (Abst.)
Vaccine and serum therapy, recent advances in, 1353 (B. Rev.)
Vaccines, comparison of relative values of intracutaneous skin test and of pathogen-selective culture in selecting bacteria for, from mixed cultures, 43[illegible] (Abst.)
Vacuum tube null indicator and glass electrode, determination of pH of normal and malignant tissues with, 1100
Vascular response to drainage of ascites, observations on, 948
Velocity factor in blood transfusion, 1206
Ventricular lesions, relationship between coronary blood supply to experimentally produced, and resulting electrocardiographic alterations, 1040
Vernes flocculation test in tuberculosis, 905 (Abst.)
Verrucous aortitis with special regard to aneurysm formation in children, 929
Vessel, combination condensing and receiving, 1116
Vitamin therapy in pulmonary tuberculosis, 966
Voge bromine tests of urine for pregnancy, five hundred and nineteen, 153

W

Wassermann and Kahn reactions upon spinal fluid in treated and untreated cases of paresis, comparison of, 546
test, study of Eagle antigens for, and flocculation test, 84
technic, a recommended, 621
Water and air balance during prolonged incubation, maintaining, 899
White blood cell dilution pipettes, calibration of, 95
Wright's blood stain, modified technic for making, 438
Wuchereria (filaria) bancrofti infection in man with an unusual history, 864

X

Xanthine diuretics, comparative study of certain, 248
X-ray, effect of salyrgan and, on rate of disappearance of thrombophlebitic edema, 243

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